The effects of sex and flexibility on indirect markers of muscle damage following a bout of eccentric exercise

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ABSTRACT

Aim: This study examined the effects of sex and flexibility on differences in serum Creatine Kinase (CK), muscle strength and muscle soreness following a bout of maximal eccentric exercise. **Method**: 10 males and 10 females were recruited for this study, none of which had performed regular resistance or flexibility training within the last year. All participants performed three baseline flexibility measures (sit and reach, standing range of motion and supine range of motion) of the knee flexors to determine levels of flexibility. Participants then performed sixty maximal eccentric contractions of their non-dominant knee flexors. Markers of muscle damage were documented by changes in maximal voluntary contraction (MVC), CK levels and muscle soreness of the knee flexor muscle group. Muscle damage markers were recorded pre (before), post (1 hour after), two, four and seven days following the eccentric exercise. **Results**: Analysis revealed a significant main effect of time on markers of muscle damage (relative knee flexor MVC torque, CK levels and muscle soreness) in both males and females following a bout of eccentric exercise. No significant main effect of sex was observed on markers of muscle damage (relative knee flexor MVC torque, CK levels and muscle soreness) following a bout of eccentric exercise. No significant association was observed between knee flexor flexibility and indirect markers of muscle damage at any time point following eccentric exercise, even when the cohort was pooled. **Conclusion**: In response to eccentric exercise there is no effect of sex or knee flexor flexibility on markers of muscle damage. The findings indicate that natural differences in flexibility, such as that seen between the sexes, has no attenuation effect on functional parameters following eccentric exercise. Further research should focus on the differences in stress/strain between the sexes and its effect on markers of muscle damage.

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1. Introduction

1.1 INTRODUCTION TO THE TOPIC

All exercise, if performed vigorously enough, can induce muscle damage amongst humans causing an impairment in muscle function over the subsequent days. Specifically, eccentric contractions are known to result in physical signs and symptoms of damage. However, it has been shown that some individuals are more susceptible than others to the physical consequences of eccentric contractions. The extent of the damage is potentially influenced by two main determinants: muscle-tendon unit (MTU) flexibility and sex (McHugh et al., 1999b, Sewright et al., 2008). Differences in these determinants attenuate markers of muscle damage and thus reduce the performance detriment seen following eccentric exercise. Nevertheless, the research regarding these determinants, specifically sex, remains contentious, perhaps due to the potential interlink between the hypothesised determinants of damage i.e. MTU flexibility and sex. The purpose of this review is to focus on how MTU flexibility and sex affect the symptoms of muscle damage at a cellular level, to review some of the current research regarding these determinants and to suggest further research to clarify the contrast within the research.

Though all vigorous exercise creates muscle damage within humans, exercise that includes a strong eccentric component (lengthening of contractile muscle against a resistance), for example walking downhill, can result in longer lasting soreness and tenderness in the days following the exercise. This is known as exercise-induced muscle damage (EIMD). The pain component of this process is often described as delayed onset muscle soreness (DOMS). Symptoms of EIMD in humans include loss of isometric and dynamic strength, muscular soreness, increased passive muscle stiffness and muscle swelling (Clarkson, 1992, Cleak and Eston, 1992). These symptoms can last up to seven days and become a detriment to performance, as exercise in the presence of muscle damage results in reduced muscle force, and greater metabolic stresses, limiting exercise intensity and duration (Gleeson et al., 1998). The term muscle damage within this review is defined as ‘the reduction in muscle function caused by the physical disruption of muscle structures involved in producing or transmitting force’ (Tiidus, 2008). Before establishing ways of attenuating muscle damage, it is important to understand why muscle damage is important in sport and exercise, and how muscle damage occurs at a cellular level.

1.2 THE IMPORTANCE OF MUSCLE DAMAGE IN SPORT AND EXERCISE

Understanding the physiological mechanisms behind EIMD is important to many health, sport and fitness professionals including athletes, therapists, physiologists and researchers alike. The knowledge of muscle damage and repair has been suggested to help prevent injury (Roig Pull, 2007), attenuate symptoms of clinical illness (Proske and Morgan, 2001) and aid sporting performance (Gulick et al., 1996, Hamill, 1991). Even though eccentric exercise is known to cause the greatest levels of muscle damage and decreases performance in the days following exercise (Clarkson, 1992), there are potential benefits of including eccentric contractions within a training regime. For example, eccentric contractions produce greater gains in hypertrophy (Farthing and Chilibeck, 2003) and, counter intuitively (see paragraph below) can result in lower indices of muscle injury in athletes (Brooks et al., 2006).

It has been reported that hamstring muscle tears are the most common acute sporting injury amongst athletes and they most often occur as a result of eccentric exercise (Sallay et al., 1996). Proske and Morgan (2001) support this with their proposal that muscle damage caused by eccentric exercise may lead to an acute muscle injury during sporting performance. Furthermore, Brooks et al. (2006) found that rugby players who included eccentric contractions into their exercise regimes were less likely to develop an injury over a season. A potential reduction in injury caused by including eccentric exercise into training regimes seen by Brooks et al. (2006) provides a benefit of performing eccentric exercise. This reduction in injury could be caused by the well-established protective effect of eccentric training on subsequent EIMD, referred to as the 'repeated bout effect' (Nosaka and Clarkson, 1995). It is reported that one bout of eccentric exercise at medium intensity could attenuate the symptoms of EIMD following a second bout. As EIMD has been suggested to result in acute muscle injuries (Proske and Morgan, 2001), reducing the symptoms of EIMD by performing eccentric exercise during exercise regimes would reduce the likelihood of an injury. The repeated bout effect causing attenuation in the performance detriments seen following eccentric exercise is a further benefit of including eccentric exercise into training regimes.

Greater gains in muscle hypertrophy and a reduction in injury likelihood, provide a compelling rationale to include eccentric training into all training regimes for athletes. Furthermore, the literature suggests that an understanding of EIMD may attenuate the damage response and the

negative impact on muscular performance. In addition, given what is known within the literature regarding sex differences in muscle damage and the understanding of extrinsic factors influencing EIMD (such as flexibility) may allow us to attenuate or understand why potential negative consequences of EIMD occur.

2. Literature Review

2.1 EXERCISE-INDUCED MUSCLE DAMAGE

2.1.1 Symptoms of exercise-induced muscle damage

Before evaluating the cellular mechanisms that are responsible for the symptoms of muscle damage, the characteristics of muscle damage and the time frame over which they occur should be established. There are a number of direct and indirect methods adopted for quantifying the extent of muscle damage, from the gold standard histological verification of damage using an electron microscope, which provides direct visual evidence of damage to the sarcomere and myofilaments, to subjective pain scales. The most common characteristics of muscle damage are functional parameters such as loss of range of motion and maximal voluntary contraction (MVC) torque; or biochemical parameters such as the presence of intramuscular enzymes or proteins in serum. As indirect markers such as: decreased MVC torque (Bowers et al., 2004), increased circulating levels of serum Creatine Kinase (CK) (Chen et al., 2011a) and increased DOMS ratings (Sewright et al., 2008) are the most common characteristics of muscle damage within humans, they will be the focus within this review. It is important to note that although CK levels are one of the most common indirect markers of muscle damage large discrepancies occur in CK levels between individuals; however, it is still considered a reliable measure of indirect muscle damage (Cleak and Eston, 1992). Most of the indirect markers of muscle damage that are reported in the literature (e.g. MVC loss and DOMS) occur immediately following eccentric exercise and subside within seven days. In contrast, there is a characteristic temporal pattern to the increase in serum CK levels which peaks four days after eccentric exercise, and then declines to pre exercise levels by approximately day seven (Figure 1). These peaks in serum CK levels occur subsequent to peaks in other markers of EIMD, with peaks in MVC force loss, and DOMS occuring within 48 hours of the eccentric exercise bout (Brown et al., 1997).

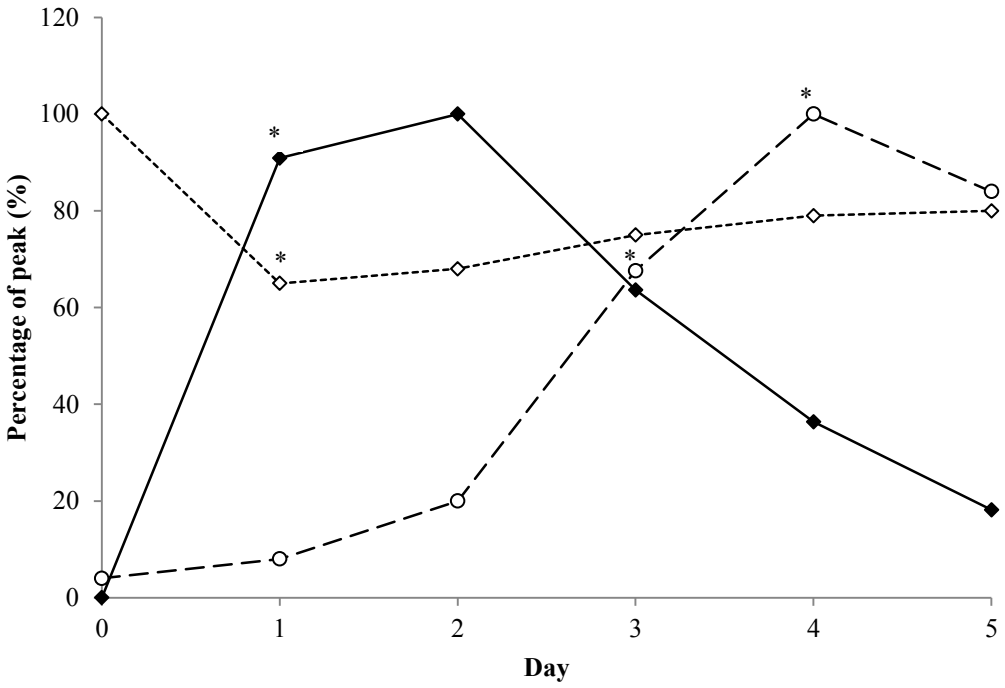

Figure 1. Creatine Kinase (Dashed line), maximum isometric contraction force of the elbow flexors (Dotted line) and soreness ratings (Solid line), expressed as percentages of peak, following a single bout of eccentric exercise. Values are means * P<0.05 vs pre-exercise (PRE). Data reproduced from Chen et al. (2011b).

2.1.2 Mechanisms of exercise-induced muscle damage

Although the time frame of symptoms associated with EIMD is well established (Brown et al., 1997, Chen et al., 2011a), there remains some contention regarding the mechanisms responsible for the functional and structural impairments. However, it is suggested that the symptoms of EIMD are caused by cellular mechanisms that occur during and following eccentric exercise (Proske and Morgan, 2001). Armstrong (1990) proposed a model of muscle damage and repair that attempts to explain the cellular mechanisms that result in the symptoms of EIMD. Armstrong (1990) suggested that muscle damage and repair occurs in four stages: initial, autogenic, phagocytic and regenerative. For the purpose of this review only the first three stages of this model will be discussed, as the cellular mechanisms within these stages describes the symptoms of muscle damage and thus the functional detriment seen following exercise (Figure 2).

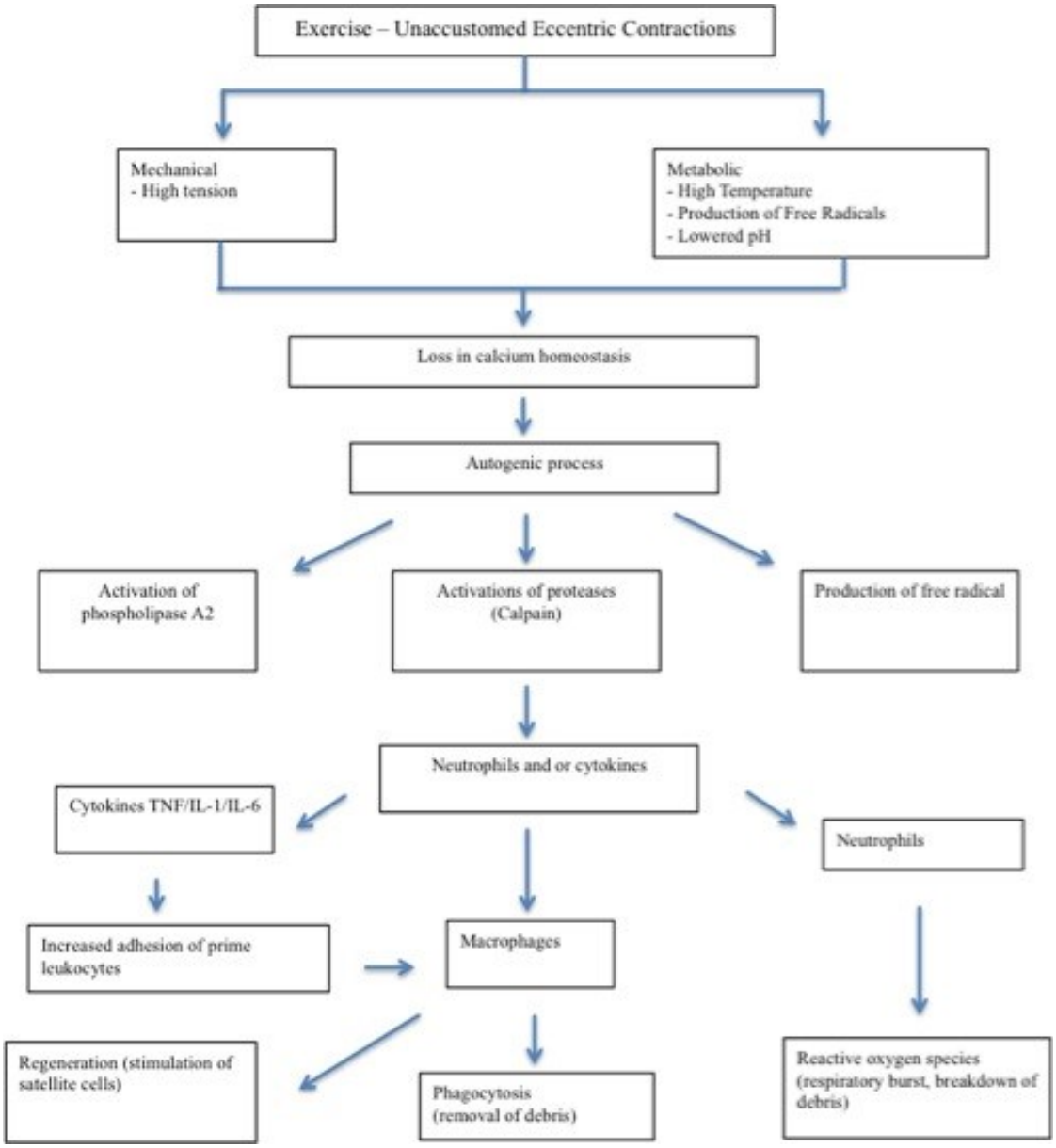

Figure 2. First three stages of the model of muscle damage and repair adapted from Armstrong (1990)

Initial event

Historically there have been numerous hypotheses for the initial damaging event associated with eccentric contractions. Although the exact nature of this event is still unknown Armstrong (1990) divided these hypotheses into two groups of mechanical or metabolic. Mechanical factors focus on the high-tension elements induced during eccentric exercise whilst metabolic factors concentrate on elements such as higher temperature, lowered pH and free oxygen radical production.

Mechanical factors

High tension within the muscle, which is generated by eccentric contractions, is suggested to cause mechanical disruption to the contractile proteins and membrane damage within the muscle cell. With the use of electron microscopes, numerous authors have observed regions of disrupted sarcomeres in the days following the repeated eccentric contractions, ranging from a single sarcomere in a myofibril, to several sarcomeres across a whole fibre in both animal (Duan et al., 1990, Lieber et al., 1991) (Figure 3) and human muscle (Newham et al., 1983). Brown and Hill (1991) also found that when muscle fibres are stretched beyond optimal sarcomere length some of the thin filaments within the sarcomeres were either partially or completely torn from the thick filaments.

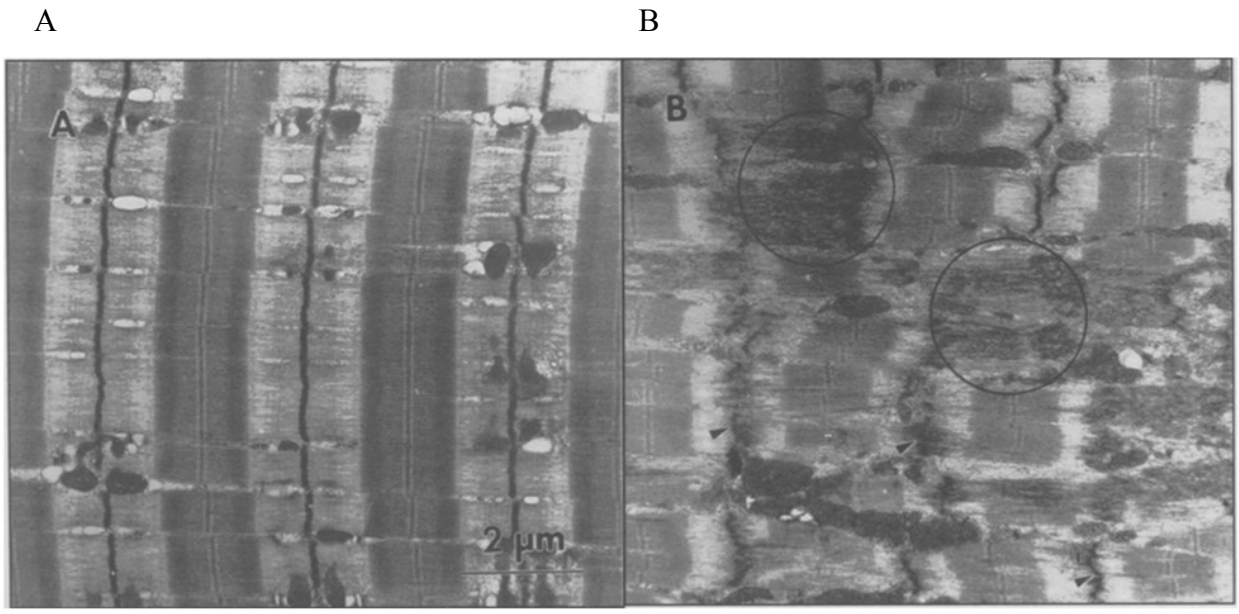

Figure 3. Longitudinal electron micrographs of rabbit tibialis anterior, subject to an isometric contraction (A) and eccentric exercise (B). B demonstrates areas of disruption i.e. the streaming and smearing of the Z-disks into A bands compared with A. (Lieber et al., 1991).

Morgan (1990) proposed the 'popping sarcomere hypothesis' which attempts to describe why high tension, created through lengthening active muscle beyond optimal length, may lead to a disruption of the cell membrane and contractile proteins. This theory holds that sarcomere disruption occurs during eccentric contractions due to failure of individual half sarcomeres creating a non-uniform lengthening within the myofibril. It is suggested that this occurs due to overstretching of the sarcomeres as the contraction occurs on the descending limb of the length-tension relationship

(Figure 4). This can create shearing of myofibrils resulting in damaged t-tubules and therefore further damage to the membrane and sarcoplasmic reticulum (Takekura et al., 2001, Yeung, 2002).

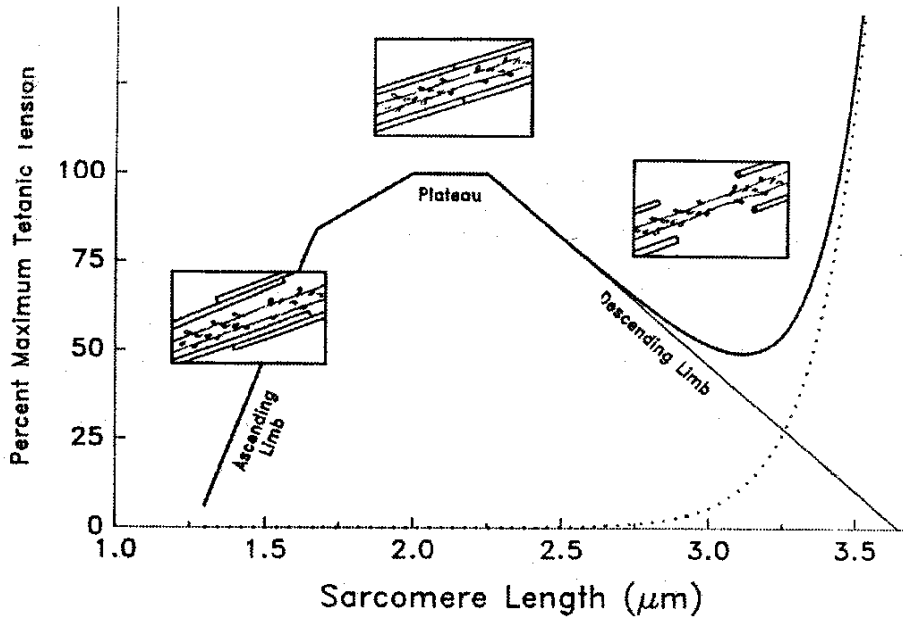

Figure 4. The sarcomere length-tension curve for frog skeletal muscle obtained using isometric contractions in single muscle fibres. Dotted line shows passive tension, continuous line shows active tension and continuous bold line shows total tension. (Lieber, 2002)

Disruption of contractile and membrane components within the muscle cells can result in a loss of calcium homeostasis through stretch-activated channels as disruptions of the cell membrane would allow calcium to move down its concentration gradient into the cell. Balnave and Allen (1995) observed significantly greater levels of intracellular calcium immediately following eccentric exercise. Disruption could also permit phospholipase A2 to physically come into contact with its phospholipid substrate in the cell membrane and lyse structural components of the sarcolemma (Palmer et al., 1983).

Metabolic factors

In comparison to the mechanical theory for the initial event, a metabolic standpoint has also been proposed. High temperatures within the muscles have been shown to have a detrimental effect on contractile proteins within muscle cells. Vasaghi-Gharamaleki et al. (2011) showed that the protein breakdown of desmin, the main intermediate filament protein in skeletal muscles, significantly increased when temperature was increased to 39°C during eccentric exercise. Furthermore, high temperatures have also been suggested to uncouple the calcium stimulated ATPase activity by

altering the fluidity of the lipid membrane surrounding the ATPase pump therefore reducing its ability to isolate calcium (Byrd, 1992). In support of this theory, higher forces are shown during eccentric contractions with greater increases in temperature compared with concentric contractions (Davies, 1979, Nadel et al., 1972). However, in contrast to this theory, authors have found symptoms of EIMD occurring in a cool muscle without the rise in temperature to 39°C. For example, Galoza et al. (2011) found an increase in CK levels and myoglobin concentrations following inter-set cooling, which consisted of applying ice packs to the muscles in intervals during the eccentric exercise. Furthermore, Vasaghi- Gharamaleki et al. (2011) found no significant effect of temperature on torque loss, a functional indicator of muscle damage, even though they found a negative effect of increased temperature on desmin. The work by Vasaghi-Gharamaleki et al. (2011) and Galoza et al. (2011) contrasts the theory that temperature is the initial event in the muscle damage process.

Free radical production has been shown to play a role in the development of muscle damage in both the initial event and the autogenic stage. It is well established that the production of free radicals or reactive oxygen species increases following lengthening contractions (McArdle et al., 2004). Free radicals are molecules which contain at least one upaired electron. (Jenkins, 1988). The unpaired electron gives free radicals their reactivity, as the unpaired electron is exchangeable. Free radicals have a destructive nature due to their oxidising effect, which makes them lethal to lipids, proteins and the extracellular matrix (Niess et al., 1999). Free radicals have been shown to oxidise the sulfhydryl groups of the ATPase pump. This phenomenon is highly correlated with a reduction in the rate of calcium uptake by the sarcoplasmic reticulum resulting in a loss of calcium homeostasis (Byrd, 1992). In support of this theory, some authors have established that antioxidants can attenuate markers of muscle damage (Bryer and Goldfarb, 2006, Taghiyar, 2013). An antioxidant is a molecule which can neutralise the unpaired electron from the free radical; for example Taghiyar et al. (2013) found that supplementation of vitamin E was able to attenuate serum CK levels following aerobic exercises. In addition, Bryer and Goldfarb (2006) found that high doses of vitamin C significantly reduced soreness for the first 24 hours following 70 eccentric elbow flexions in healthy males. It is believed that antioxidants are able to reduce the reactive oxygen species, resulting in reduced oxidative stress upon the tissues and thus less muscle damage (Goldfarb, 1999). Although antioxidants have been shown to have some effects on reducing symptoms of muscle damage, this is not conclusive (Connolly et al., 2006, Thompson et al., 2001). For example, Thompson et al. (2001) and Connolly et al. (2006) found no significant effects of vitamin C supplementation on CK levels, torque loss or DOMS following eccentric exercise retrospectively.

Lowered pH from an increase in hydrogen ions, has also been shown to affect the sarcoplasmic reticulum's ability to uptake calcium. It is suggested that this is due to hydrogen and calcium ions competing for the calcium-binding site on the ATPase pump (Byrd, 1992). Increased temperature, free radical production and lowered pH all result in a loss in calcium homeostasis within the muscle cells. Although Armstrong divided these two potential initial mechanisms it is likely, based on recent research, that they occur concurrently with the initial mechanism of damage leading to the autogenic response associated with the clearance and repair of damage muscle cells.

Autogenic stage

A loss of calcium homeostasis is evident within both the metabolic and mechanical hypothesis for the initial event. The role of calcium within the damage and repair process is an important factor in the development of the symptoms observed following eccentric exercise. Research has shown that inhibiting the flux of calcium across the sarcoplasmic reticulum reduces the indices of muscle damage in rodents (Byrd, 1992). Jackson, Jones and Edwards (1984) proposed that increased calcium results in the activation of the enzyme Phospholipase A2. The activation of this enzyme can have damaging effects on the cell membrane through the production of detergent fatty acids and lysophospholipids. Damage to the cell membrane can result in membrane fluidity and loss of intracellular enzymes such as CK. Supporting evidence has shown that inhibiting Phospholipase A2 significantly reduces the loss of intracellular enzymes into the blood stream (Jenkins, 1988) . Damage to the cell membrane can create impairment of the E-C coupling system and ultimately result in the decline in tension observed following eccentric exercise (Brown et al., 1997). Warren et al. (1993) found that caffeine, which directly releases calcium from the sarcoplasmic reticulum and bypasses some of the elements of the E-C coupling system, was able to recover the decrease in force seen following exercise. This therefore suggests that the decrease in force was due to failure in a step in the E-C coupling process.

In addition to the proposal that calcium activates Phospholipase A2, increased levels of calcium have also been shown to stimulate proteases. It is suggested that these proteases act directly on the proteins within the cell membrane (calpain) and structural components of the muscle fibres (Belcastro et al., 1998). Observations by Liber, Thornell and Friden (1996) have shown that the amount of desmin was reduced following a bout of eccentric exercise. Degradation of structural proteins, such as desmin, would result in a reduction in the ability of the muscle fibre to transmit force laterally to other fibres. In addition, damage to the contractile proteins and connective tissues

has been suggested as a theory behind the pain experienced following eccentric exercise. Nociceptors situated in the muscle connective tissue and musculotendinous junction are stimulated when the contractile proteins and connective tissues are damaged resulting in the pain sensation (Cheung et al., 2003).

Phagocytic stage

Damaged tissues initiate the activation of the acute phase response sometimes known as the inflammatory response. This stage can start between 4-6 hours following the exercise and last up until 4 days following (Pizza et al., 2002). This stage works to primarily facilitate antibacterial responses before clearing debris and tissue fragments from the damage tissues. Two cell populations are required to aid this process: inflammatory cells used to remove debris and myogenic cells used to replace damaged muscle tissues. Cytokines, produced by circulating and tissue resident leucocytes (white blood cells), are used to transport these important cells into the damage tissues (Pyne, 1994). Cytokines are small polypeptides that act as intracellular mediators in the generation of the immune response. Interleukin (IL)-1, interferon, IL-2, IL-6 and tumor necrosis factor-α (TNFα) are the cytokines used for the inflammatory process. TNFα and IL are suggested to prime leucocyte function and macrophage activation (MacIntyre et al., 1995). Tidball (1995) found increases in the concentrations of IL-1 following damaging exercise supporting the importance of these cytokines in the muscle damage and repair process. In addition to cytokines, leucocytes specifically neutrophils and monocytes/macrophages play a role in the phagocytic stage. These cells have three main roles in the inflammatory response: 1) attack and breakdown of debris, 2) removal of cellular debris and 3) regeneration of cells. It is suggested that the inflammatory process creates the symptoms of pain and soreness in the days following the eccentric exercise. Inflammatory cell infiltration and oedema formulation in the injured cells creates an osmotic pressure that activates a group of sensory neurons (IV), resulting in the pain experienced (Friden et al., 1986).

Although the exact mechanisms of cellular disruption remain unclear, there is a pattern of events that appears to occur. Following the initial disruption of the sarcomere as a result of eccentric contractions, there is a secondary metabolic phase which results in further structural damage, and a third inflammatory phase associated with the clearance of debris. In regards to the implications on the extent of the muscular disruption, there are a number of determinants, for example the presence of antioxidants, which can play a role in determining the extent of EIMD following eccentric contractions.

2.2 DETERMINANTS OF EXERCISE-INDUCED MUSCLE DAMAGE

Research has shown that manipulating determinants of muscle damage could reduce the symptoms of EIMD (Chen et al., 2011a, McHugh et al., 1999b). One particularly interesting determinant of muscle damage is flexibility. One way flexibility has been linked with damage is with acute interventions that manipulate flexibility. It is well known that performers have been advocated to perform pre-exercise stretching with the aim of reducing local muscular injury (Cheung et al., 2003) and that static stretching increases ROM (Magnusson et al., 1998). However, there remains no evidence that static stretching has a significant impact on markers of muscle damage; for example Torres et al. (2013) found no effects of post eccentric exercise stretching on markers of muscle damage (CK levels, muscle soreness and maximal concentric torque). Furthermore, Nameni (2011) found no effects of 20 minutes of pre-exercise static stretching on muscle soreness following eccentric exercise.

Following evidence that pre-exercise stretching has little or no protective effects on the markers of muscle damage (High et al., 1989, Johansson et al., 1999, Nameni, 2011, Torres, 2013), authors have turned their attention to the role of MTU flexibility and long term flexibility training on attenuating EIMD. In addition to this, an interlinking determinant to flexibility that has caught the attention of many authors is sex, specifically its association with the degree of isometric force loss, increased CK levels and soreness. This is an interesting determinant as it still remains contentious, perhaps due to the fact that its effect may be interlinking with an individual's intrinsic flexibility. The next three sections of this review focus on the determinants of muscle damage sex and flexibility, how they are suggested to reduce markers of muscle damage (particularly isometric force loss, increase CK levels and increase soreness) and the current research regarding these determinants.

2.2.1 The role of flexibility in attenuating muscle damage

Morgan's (1990) popping sarcomere hypothesis, as explained previously, plays a key role in the understanding of the suggested mechanism behind the protective effects of increased muscle tendon unit (MTU) flexibility on EIMD. The key component of this hypothesis is that the disruption of sarcomeres occurs on the descending limb of the length tension relationship. It has previously been shown that changing or shifting the optimal angle of force generation to a longer muscle length can attenuate the symptoms of EIMD (Lynn et al., 1998) (Figure 5). Proske and Morgan (2001)

proposed that a shift in optimal angle could be representative of an increase in sarcomeres in series. Supporting evidence by Lynn et al. (1998) has shown that training the muscle at a longer muscle length, such as through decline running, increased the amount of sarcomeres in series and thus caused the attenuation in EIMD following lengthening contractions. In addition, Chen et al. (2009) found that following a bout of flexibility training there was a shift in the optimal angle for maximum force production towards longer muscle lengths, which suggests flexibility training could result in an increase in sarcomeres in series and potentially less EIMD. In accordance with Morgan's popping sarcomere theory, it could be postulated that flexibility can attenuate muscle damage through an increase in sarcomeres in series. This would therefore decrease the length of a sarcomere at any given joint angle and avoid the potential damaging increase in strain associated with contractions on the descending limb of the length tension curve.

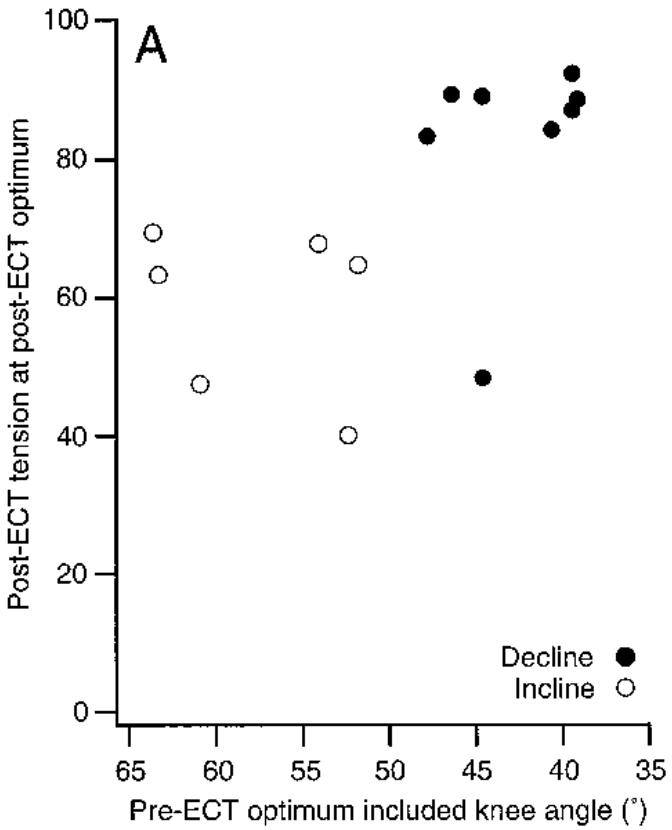

Figure 5. The effects of a series of eccentric contractions on tension measured at post eccentric optimal (post-ECT optimal) in decline running trained rats (closed circles) and incline running trained rats (open circles) (Lynn et al., 1998).

In support of the suggestion that flexibility attenuates muscle damage, McHugh et al. (1999b) concluded there was a significant attenuation in markers of muscle damage, specifically isometric

knee flexor MVC torque loss and perceived soreness, in 'compliant' compared with 'stiff' muscles (Figure 6). Muscle compliance was determined through passive stiffness of the hamstring muscles, which has been correlated with other measurements of flexibility such as the sit-and-reach test (Magnusson et al., 1997). Flexibility training further highlights the attenuation effects of flexibility of the MTU on markers of muscle damage. Eston et al. (2007) reported that eight weeks of proprioceptive neuromuscular facilitation (PNF) stretch training, performed twice a week, enhanced torque recovery at longer muscle lengths resulting from EIMD. However, they found no differences at shorter muscle lengths. In addition to this, Chen et al. (2011a) found that following eight weeks of PNF training there was a significant decrease in markers of muscle damage (CK activity, knee flexor isometric MVC torque loss and perceived muscle soreness) compared with an age matched control group (i.e. no flexibility training). Chen et al. (2011a) also observed a shift in optimal angle towards a longer muscle length and an increase in range of motion (ROM) following the flexibility training. These studies therefore provide evidence that increased flexibility through training can attenuate markers of muscle damage.

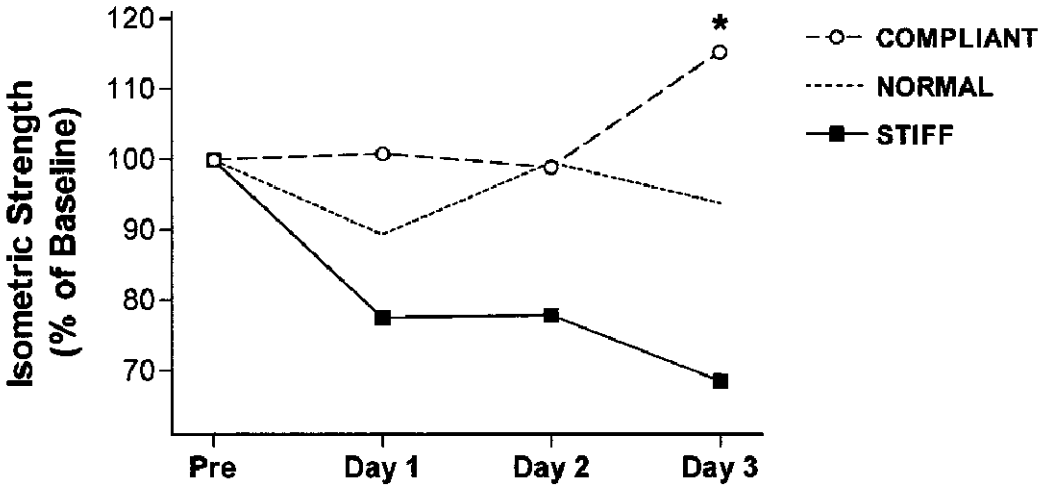

Figure 6. Maximum isometric contraction force of the hamstring muscles. Values are means (SD) * P<0.05 compared with pre-exercise values (PRE). (McHugh et al., 1999b)

Even though previous research provides experimental evidence that increased compliance of the MTU attenuates the symptoms of EIMD, the limitations within the research (see below) do not allow for a causal relationship or significant conclusion to be made regarding the role of flexibility and EIMD. For example, in their investigation of the role of flexibility on strength loss following EIMD, McHugh et al. (1999b) inadvertently introduced a sex bias when grouping the participants based on MTU compliance, with six of the seven participant's in the compliant group being female

and six of the seven participants in the stiff group being males. By allocating a sex bias into their groupings, McHugh et al. (1999b) were unable to distinguish the flexibility effect from inherent differences in the MTU associated with naturally higher levels of oestrogen in the female participants, which has been shown to increase tendon compliance (Kubo et al., 2003). Any change in tendon properties may have specific influences on EIMD beyond the passive flexibility of the muscle. In addition, muscle cross-sectional area has been positively correlated with mid range MTU stiffness (Magnusson et al., 1997) and thus could explain the sex bias within the stiffness groupings. However, this again would not allow for the conclusion that greater stiffness equals greater muscle damage as there is no evidence that it is not simply a sex difference that creates the attenuation. Furthermore, McHugh et al. (1999b) only measured serum CK levels in 12 subjects of mixed sex, classified into three levels of flexibility (five compliant, four normal and three stiff), which makes the results susceptible to the wide variability associated with the post eccentric strength response. Chen et al. (2011a) also only used male participants within their study. The use of only male participants is not representative of the whole population and therefore the findings of this study may not be imitated within females.

The lack of sex-controlled studies within the literature makes it impossible to determine how sex affects the interaction between flexibility and muscle damage. Females are significantly more flexible than males due to differences in MTU properties (Gajdosik et al., 1990). It is therefore possible that sex differences in MTU properties account for the lower indices in muscle damage, thus supporting the theory that increased compliance can attenuate symptoms of muscle damage in females. However, it is also possible that a protective effect of oestrogen (see page 15), a hormone found in much higher quantities in females compared with males, could also create the reduced indices of muscle damage.

2.2.2 The role of sex in attenuating muscle damage

To explore the hypothesis that sex has an effect on muscle damage through the protective effects of oestrogen, it is important to review the research regarding sex and oestrogen and its effects on markers of muscle damage. It is proposed that sex can influence the degree of muscle damage and inflammation (Sewright et al., 2008). Females have been shown to have significantly lower structural and enzyme markers of muscle damage compared with males following a bout of eccentric exercise (Amelink and Bar, 1986, Komulainen et al., 1999, Sewright et al., 2008).

Animal Models

Animal research has established that there is a significant attenuation in markers of muscle damage, specifically CK and inflammatory markers, following a bout of eccentric exercise in females compared with males (Amelink and Bar, 1986, Komulainen et al., 1999, Salminen and Kihlstrom, 1985). Komulainen et al. (1999) found that for up to 96 hours following eccentric exercise (downhill running), male rodents exhibited greater indices of muscle damage, specifically damage to structural proteins and fibre swelling, compared to female rodents. In addition, it has been noted that levels of B-glucuronidase, an enzyme activated in response to muscle disruption, were significantly greater in males rodents compared with females (Salminen and Kihlstrom, 1985). The evidence that females have an attenuated response in muscle damage compared to males stimulated the hypothesis that hormones, specifically oestrogen, may create a protective effect against muscle damage.

Oestrogen and muscle damage

Oestrogens are a group of 18 carbon steroids secreted primarily from the ovaries and adrenal glands in females. Oestrogen is also secreted by the testes and adrenal glands in males but to a much lesser extent than females (Bunt, 1990). The possible protective role of oestrogen can occur through three pathways: 1) protection against oxidative damage 2) membrane stabilising properties or 3) a gene regulatory effect. Oestrogen has a high antioxidant capacity offering an additional line of defence against oxidative damage in females. As previously mentioned, antioxidants offer protection through neutralising the unpaired electron from the free radical species (Taghiyar, 2013). In addition to this, it is believed that oestrogen has membrane stability properties through its interactions with the phospholipid bilayer, creating less membrane fluidity (Wiseman and Quinn, 1994). Lastly, it has been shown that oestrogen could reduce the gene expression of pro-inflammatory cytokines through its strong antioxidant properties (Beato, 1989). Previous research has shown that antioxidants such as tocopherol can slow the inflammatory process by inhibiting the gene expression of pro-inflammatory cytokines(Yoshikawa and Yoshida, 2000). Therefore, oestrogen could possibly reduce further damage by reducing infiltration of cells such as neutrophils, however in doing this could inhibit the repair process. It is not well established which method of protection (if any) oestrogen offers against exercise induced muscle damage due to the complex nature of the muscle damage and repair model. However, it is accepted that oestrogen appears to be associated with an attenuation in markers of muscle damage in animals.

Shumate et al. (1979) first established that oestrogen significantly attenuated markers of muscle damage with findings that female rodents had significantly lower levels of circulating CK and membrane disruption compared with males. In further support of this, Amelink et al. (1990) found

significantly lower levels of CK in male, female and ovariesctomized rodents when the muscles were isolated and treated with oestrogen supplementation; therefore showing that oestrogen has an attenuating effect on indirect markers of muscle damage. Discrepancies in levels of B-glucuronidase have also been noted in ovariectomized female rodents who have had oestrogen replacement therapy with findings of significantly lower levels of B-glucuronidase (a marker of muscle disruption) compared with ovariectomized female rodents (Enns and Tiidus, 2008). Not only has oestrogen been shown to attenuate markers of muscle damage, it has also been shown to enhance the potential for muscle repair. Enns, Iqbalm Tiidus (2008) recently found that oestrogen supplementation in female rodents could enhance activation of satellite cells following downhill running, which are essential to the muscle repair process (Charge and Rudnicki, 2004). Although there is a body of evidence that demonstrates that sex has a significant effect on markers of muscle damage within animals, the research within human is limited and still remains contentious.

Human Models

In support of the sex differences in markers of muscle damage, a number of studies, using running or eccentric exercise, have found significantly greater increases in serum CK levels in males compared with females (Sewright et al., 2008, Stupka et al., 2001) (Table 1). Sewright et al. (2008) found a significantly greater increase (3%) in CK levels following 50 maximal contractions of the elbow flexors, in males compared with females on the fourth day following the eccentric exercise. Stupka et al. (2001) also found a significantly greater increase (405%) in CK levels in males compared with females even though they found no differences in Z-disk streaming, which is a direct characteristic of ultrastructure damage. These findings partly support animal data however, in contrast, some studies have found no significant difference in markers of EIMD between the sexes (Dannecker et al., 2012, Sayers and Clarkson, 2001, Stupka et al., 2000) (Table 1). Stupka et al. (2000) found no significant difference between males and females in serum CK levels or Z-line streaming following unilateral leg press exercises. In addition to this, Sayers and Clarkson (2001) investigated force loss and recovery following 50 maximal eccentric contractions of the elbow flexors and found no significant differences between the sexes.

Table 1. Sex differences in markers of muscle damage

Author	Subjects	Damage protocol	Muscles used	Markers of muscle damage analysed	Results
Stupka et al., 2000	8 males and 8 females. All females were oral contraceptive users.	3 sets of 12 eccentric unilateral leg press and 9 sets of 12 eccentric unilateral leg extension with 1 minute between sets.	Knee extensors	Plasma granulocyte counts, CK and Z-line streaming	Significantly greater plasma granulocyte counts in males compared to females at 48hrs. No significant differences in CK or Z-line streaming between the sexes.
Stupka et al., 2001	8 males and 8 females. 5 of the 8 females were oral contraceptive users.	3 sets of 12 eccentric unilateral leg press and 10 sets of 10 eccentric unilateral leg extension with 3 minutes between sets.	Knee extensors	Loss in MVC, CK, Neutrophil and macrophages counts and Z-line streaming.	Significantly lower CK activity in females compared with males. No significant differences in Z-line streaming, loss in MVC or macrophage and neutrophil counts between the sexes.
Sayers & Clarkson, 2001	98 males and 94 females. No mention of oral contraceptive use.	2 sets of 25 maximal eccentric contractions with 5 minutes rest between sets.	Elbow flexors	Loss in MVC	No significant differences in MVC loss between males and females.
Sewright et al., 2008	42 males and 52 females. 28 of the 52 females were oral contraceptive users	2 sets of 25 maximal eccentric contractions with 5 minutes rest between sets.	Elbow flexors	CK, MB, loss in MVC and muscle soreness.	Significantly lower CK activity in females compared to males. Significantly greater loss in MVC immediately following

					eccentric exercise compared with males. No significant differences in soreness or MB between the sexes.
Dannecker et al., 2012	19 males and 14 females. 2 of the 14 women were oral contraceptive users.	3 sets of 12 maximal isokinetic eccentric contractions (90 degrees per second).	Elbow flexors	Soreness, arm girth, resting elbow extension, isometric elbow flexion strength, MB, tumor necrosis factor (TNFa), interleukin 1beta (IL1b) and total nitric oxide (NO).	No significant differences in any of the markers of muscle damage.

Limitations within the human model literature

From the previous research there are three possible limitations that may justify the contention within the literature: 1) lack of oestrogen control and measurement, 2) timing of the measurements, and 3) flexibility acting as a confounding variable. Some authors have neglected the potential effects of oestrogen on markers of muscle damage by not controlling for the use of oral contraceptives and the menstrual cycle phase when measurements were taken (for example Sayers & Clarkson, (2001)). Discrepancies in oestrogen throughout the testing could have affected the results as oral contraceptive users have a significant down-regulation in endogenous oestrogen compared with non-users (Bryant, 2008) and as previously shown, oestrogen can attenuate markers of muscle damage (Shumate et al., 1979). Therefore, the use of oral contraceptives may have influenced the results through the reduction in endogenous oestrogen production and thus reduced the attenuation in muscle damage previously seen within females. In addition to this, some authors have not measured oestrogen or measured oestrogen at different points throughout the menstrual cycle. It is possible to say that there would be a difference in levels of oestrogen between males and females due to the nature of their regulatory hormones. However, as oestrogen levels are not reported it cannot be assumed that the difference was large enough to create differences in all the indirect markers of muscle damage.

Timing of the measurements throughout the testing may have also lead to a lack of significant differences between the sexes as some authors did not take measurements at times where peaks in markers of muscle damage occur. For example, it is well established that the peak in muscle soreness and isometric force loss occurs approximately 48 hours following eccentric exercise (Chen et al., 2011a); however, some authors (such as Sewright et al., (2008)) did not measure at this time point. In addition, Stupka et al. (2000) only took measurements before the eccentric exercise, 24 hours, 48 hours and six days following the eccentric exercise. Stupka et al. (2000) may not have observed a peak in serum CK levels because CK peaks on the third/forth day following the exercise (Brown et al., 1997, Chen et al., 2011a).

Even though there is a body of research which suggests that flexibility has an impact on markers of muscle damage (Chen et al., 2011a, McHugh et al., 1999b), no authors have included this as a co-variate within their testing procedures. Flexibility may play a key factor in the findings of significant and non-significant differences between the sexes in markers of muscle damage as females have greater flexibility than males (Soucie et al., 2011). It is therefore possible that the flexibility of the participant may have led to the findings that females are less susceptible to muscle damage.

Alternatively, it is also possible that the male participants used within the studies have greater or similar amounts of flexibility compared to females leading to the lack of difference. In addition to this, oral contraceptive users have been shown to have significantly greater tendon stiffness than non-oral contraceptive users, which has been suggested to be caused by long-term suppression of oestrogen (Bryant, 2008). Therefore, the use of oral contraceptives users to examine the effects of sex on muscle damage may have influenced the findings.

In conclusion, there is a lot of animal research supporting the notion that females are less susceptible to muscle damage compared to males. However, the human research remains inconclusive. Due to the findings of the effects of flexibility on muscle damage research and the limitations in the effects of sex on muscle damage, it is possible that flexibility is acting as a confounding variable within humans which would result in the conflict in the sex research.

2.3 SEX DIFFERENCE IN MUSCLE AND TENDON PROPERTIES

In addition to a direct role of oestrogen attenuating markers of EIMD in females, it is possible that differences in the morphological and elastic properties may also contribute. As mentioned previously, there are a number of studies that have suggested a potential role of tendon and muscle compliance or flexibility on attenuating EIMD (Chen et al., 2011a, McHugh et al., 1999b). Indeed when the determinants of flexibility are considered, any attenuating role of flexibility on muscle damage may be attributable to the co-variates of flexibility or MTU compliance. Magnusson et al. (1997) proposed that the determinants of musculoskeletal flexibility are based on three factors: viscoelastic properties of the MTU, cross sectional area (CSA) and stretch tolerance. It is well known that these parameters differ between males and females (Blackburn et al., 2004b, Hoge et al., 2010, Kanehisa et al., 1994) and this could be the reason why females are more flexible and thus are less susceptible to muscle damage than males.

Viscoelastic properties between males and females

It is well documented that males have significantly greater MTU stiffness compared with females when stiffness is assessed using the passive torque angle relationship (Blackburn et al., 2004a, Gajdosik et al., 1990). Morse (2011) furthered this notion with findings that males have significantly greater *Gastrocnemius medialis* (GM) muscle stiffness compared with females when assessed separately to the tendon using distal displacement of the muscle tendon junction (MTJ). In addition to this, it has been reported that females differ significantly from males in the viscoelastic properties of

the tendon, with findings of lower tendon stiffness and hysteresis in females (Kubo et al., 2003, Onambele et al., 2007). Authors suggest that hormones such as oestrogen contribute to the differences of MTU stiffness seen between males and females (Kjaer and Hansen, 2008) however, this topic is still in debate. It is proposed that oestrogen receptors are found in fibroblasts of tendons and ligaments and thus may alter collagen synthesis and the tissues behaviour (Kjaer and Hansen, 2008). In vitro testing of human tendons suggests that oestrogen significantly reduces collagen fractional synthesis rates in the tendon (Miller et al., 2007), providing experimental evidence that hormonal regulation affects the stiffness of human tendons. In support of this, Bryant et al. (2008) found a significant increase in stiffness of the Achilles tendon following long-term suppression of oestrogen via oral contraceptive use. However, some authors such as Burgess, Pearson and Onambele (2009) found no significant differences in female medial gastrocnemius tendon throughout the menstrual cycle where significant fluctuations in oestrogen occur.

Cross sectional area

In addition to differences in the viscoelastic properties of the muscle and tendon between the sexes, differences in muscle CSA also exist. It is well established that males have significantly greater muscle CSA compared to females (Kanehisa et al., 1994). Magnusson (1997) found that CSA of the hamstrings was significantly related to the mid portion of the stiffness curve, but not the initial or final portion of the curve. It is suggested that greater CSA would contribute to passive muscle stiffness through greater viscoelastic resistance to stretch from a greater area for the distribution of passive forces, consistant with the viscoelastic principles of tendon elongation (Magnusson et al., 2003). This would suggest that CSA does contribute to mid-range stiffness however, other factors such as stretch tolerance are likely to contribute to factors such as endROM and thus the final portion of the curve (Magnusson et al., 1997).

Stretch tolerance

The final determinant of flexibility proposed by Magnusson et al. (1997) is stretch tolerance. Hoge et al. (2010) found that 20 minutes of passive stretching significantly increased ROM in women but not in men, with no significant changes in MTU stiffness. This is consistent with work by Magnusson et al. (1998) who also found increases in ROM following static stretching but no differences in MTU stiffness. The authors attribute the findings to differences in short-term stretch tolerance rather than differences in viscoelastic properties, suggesting females have a greater stretch tolerance than males. An increase in stretch tolerance during stretching and thus flexibility in females would provide an explanation as to why females are less susceptible to muscle damage.

There are three key factors that determine musculoskeletal flexibility and each of these factors differ between the sexes. Greater muscle and tendon stiffness, greater CSA and reduced stretch tolerance could explain why males are less flexible, and thus more susceptible to muscle damage compared with females; assuming that flexibility equates to less sarcomere strain during eccentric contractions.

Following the evidence that the properties of the MTU and thus flexibility differ substantially between males and females, possibly owing to oestrogen's suppression of collagen synthesis, it is plausible that females are less susceptible to muscle damage due to their increased flexibility. However, due to the limitations within this research it remains inconclusive what the effect of sex on muscle damage are, and how sex differences in flexibility effects this interaction.

3. Rationale, aims and hypothesis

3.1 RATIONALE

Even though animal research shows that there is a sex differences in markers of muscle damage the human research remains contentious. This could be owing to the limitations within the literature such as the lack of oestrogen control or measurement of flexibility. As females are significantly more flexible than males, due to differences in MTU properties (Gajdosik et al., 1990), it is possible that sex differences in MTU properties contributes to the lower indices in muscle damage, thus supporting the theory that increased compliance can attenuate symptoms of muscle damage (Chen et al., 2011a, McHugh et al., 1999b). However, research also suggests that oestrogen provides a protective effect against markers of muscle damage thus supporting that of a sex difference not simply a flexibility difference. Due to the limitations within the literature is impossible to decipher whether there is a sex difference that creates the attenuation in markers of muscle damage, whether it is a difference in muscle tendon properties that is naturally seen between the sexes or an interaction between the two.

3.2 AIM

Therefore the aim of the present study was to determine the effects of sex and sex differences in flexibility on indices of muscle damage, specifically CK levels, ability to generate force and perceived soreness, following a bout of eccentric exercise.

3.3 HYPOTHESES

1. Lower indices of muscle damage (CK levels, MVC loss and soreness rating) will be observed in females compared with males.
2. There will be a significant negative correlation between muscle damage and flexibility within males and females.
3. If the first two hypotheses are proven correct there will be an interaction effect between the effects of sex and flexibility on markers of muscle damage.

4. Method

4.1 STUDY DESIGN AND METHODS USED

4.1.1 Participants

Ten males (age 21 ± 1 years, stature 1.76 ± 0.10 m, mass 77.0 ± 12.0 kg) and ten females (age 21 ± 2 years, stature 1.64 ± 0.35 m, mass 62.3 ± 6.6 kg) volunteered to participate in this study. Participant demographics are displayed as means ± SD. Participants reported no regular resistance or flexibility training within the last year and no muscle, bone or joint injuries to the lower extremities. None of the participants had any known (local or systemic) neurological, musculoskeletal, inflammatory or metabolic disorders. Participants self-reported as "moderately active" (i.e. undertaking less than two hours of structured physical activity per week). None of the females within the study were taking any form of hormone-based contraceptives. All participants gave written informed consent and all procedures were approved by the institutional Ethics Committee of Manchester Metropolitan University.

4.1.2 Study Design

Prior to any testing procedures participants were asked to complete a confidential questionnaire to determine their physical activity levels, lifestyle habits, and for females only, menstrual cycle and pill use. Females were tested as close a possible (± 2 days) to the 14th day of their menstrual cycle (proliferative phase), as this is the point in the menstrual cycle when oestrogen is at its highest. All participants completed the same testing procedure and were required to attend the laboratory on six separate occasions. The first three sessions included familiarisation, resting measurements and eccentric exercise whilst the last three included follow up measurements (Figure 7).

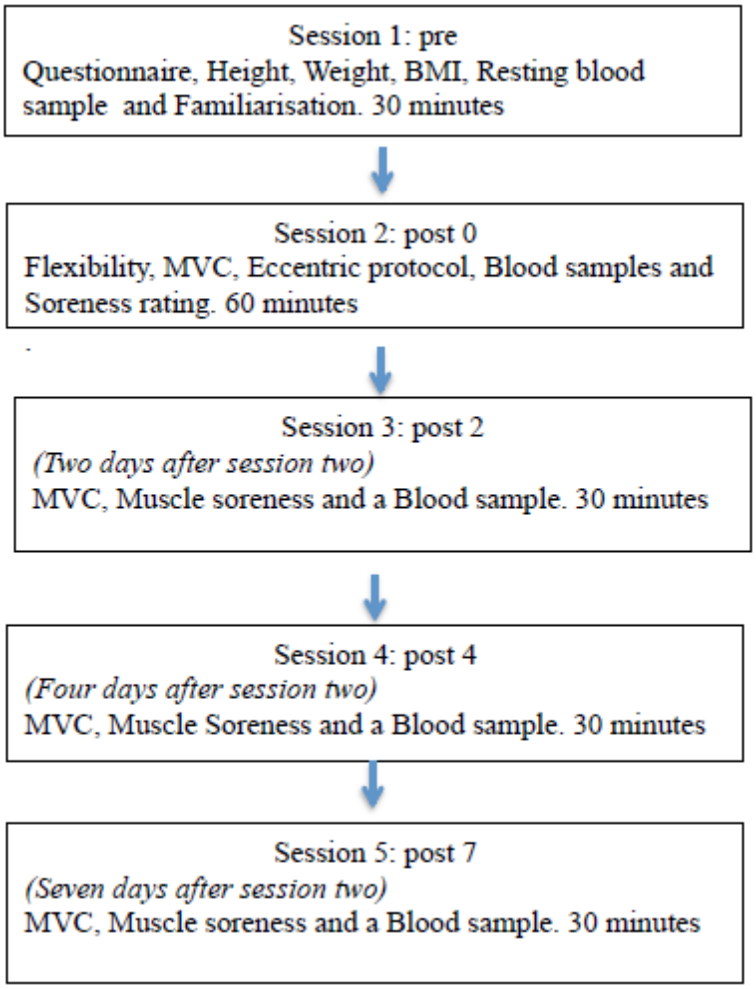

Figure 7. Timeline of testing procedures

4.1.3 Isometric Maximal Voluntary Contraction Torque and Optimal angle

Participants were seated in an upright position on an isokinetic dynamometer (Cybex Norm, Cybex International Inc., NY, USA) with their shoulder and hips strapped to the chair to prevent hip flexion. The non-dominant leg was secured to the dynamometer arm at the ankle, ensuring that the knee joint was aligned with the axis of rotation of the dynamometer. The dynamometer was locked at a 45° angle and weighed to allow for gravity correction of limb mass. Participants were then instructed to conduct a warm up by performing a series of 5 sub-maximal isokinetic ($60^{o}\ s^{-1}$) flexions and extensions of the leg at the knee joint. Following the warm up, the optimal angle of isometric knee flexion MVC torque was found. Participants performed knee flexor MVC's (4 seconds each) at six randomized angles of knee flexion (20^{o}, 30^{o}, 40^{o}, 50^{o}, 60^{o} and 70^{o}). Previous research has shown that determining knee flexor MVC using an isokinetic dynamometer is reliable (Molczyk and Eickhoff, 1991). Participants performed MVC's twice at

each angle with two minutes rest between each contraction to prevent development of fatigue. Verbal encouragement was given to maximize all MVC's. The highest torque throughout a contraction was recorded and the highest value of the two contractions was deemed as MVC.

4.1.4 ROM/Flexibility

Flexibility of the hamstrings and ROM about the hip joint was measured in three positions: 1) standing, 2) supine and 3) sit and reach. The standing position endROM was measured whilst the participant was standing, with the hip joint of the non-dominant leg aligned with the axis of rotation of the dynamometer and the leg secured to the dynamometer arm at the knee (Figure 8). Prior to the stretch the non-dominant leg was braced in full extension to prevent knee flexion and extension during the movement. Passive end range of motion (endROM) was volitional and assessed using a similar method to that of McHugh et al. (1998). This involved passive isokinetic hip flexion from 0° (vertical femur) at $1 \cdot s^{-1}$ until mild discomfort was experienced and expressed by the participant. The angle at which this occurred was deemed endROM. This was repeated three times, taking the highest value as endROM. Previous work has shown this method of determining endROM as reliable (Chen et al., 2011a). The second position was measured in a similar fashion however, participants were asked to lie in a supine position on the isokinetic dynamometer. The hip joint of the non-dominant leg was aligned with the axis of rotation of the dynamometer; the leg was braced in full extension and secured to the dynamometer arm at the knee. endROM was determined in the same way as the standing position which involved passive isokinetic hip flexion from 0° (horizontal femur) at $1 \cdot s^{-1}$ until mild discomfort was experienced and expressed by the participant. This procedure was repeated three times, taking the highest value as endROM. The final measure of flexibility was assessed using a sit and reach test. Participants were asked to sit with their legs fully extended in front of them. Shoes and socks were removed and the soles of their feet were placed against the sit and reach box. Participants were instructed to reach forward and touch as far along the box as possible without bending their knees or leaning to one side. The procedure was repeated three times. This test is a well-established protocol for measurement of hamstring and lower back flexibility. Previous research has deemed this test reliable and valid (Ayala et al., 2012, Gabbe, 2004). Individual united weighted composite for flexibility was calculated for each participant in order to create one flexibility value. Z-scores of the three flexibility measures were calculated and added together to create this. Using this method eleven participants were catagorized as flexible and nine participants were catergorized as non-flexible.

Figure 8. Standing ROM using an isokinetic dynamometer. Knee is strapped in full extension using a knee splint.

4.1.5 Anatomical Cross sectional area (ACSA)

ACSA of the *Bicep Femoris*, *Semitendinosus* and *Semimembranosus* (Knee flexor ACSA) was determined using a fixed 0.2 Teslar MRI scanner (E-Scan, ESAOTE Biomedica, Genova, Italy). Before the scan participants were required to lay supine for 15 minutes to control for a shift in fluid that occurs when moving from an upright to supine position (Berg et al., 1993). To ensure consistency between participants each scan was positioned at 50% of femur length. This was established as 50% of the distance between the greater trochanter and the lateral femoral tuberosity identified using B-Mode ultrasonography (ATL-HDI 3000, Bothell, USA). A low density reference marker was placed on the skin at 50% of femur length. A coronal localizer scan was first performed to ensure the image was in line with the low-density marker and to align the scan perpendicular to the femur. Then five serial, equidistant, transvers T1-weighted MRI images were recorded (TR=615 ms, TE=16 ms, field of view = 1145 x 584, matrix = 192 x 208, slice thickness = 7mm, gap = 1 mm) with a total duration of 5 minutes. The boarders of the separate muscle were traced and ACSA was calculated with DICOM imaging and analysis software (OsiriX medical imaging software, OsiriX, Atlanta, USA).

4.1.6 Eccentric Exercise

Eccentric exercise was performed on an isokinetic dynamometer (Cybex Norm, Cybex International Inc., NY, USA), in the same seated position as described for the isometric maximal voluntary contraction. Participants were required to perform six sets of 10 maximal lengthening contractions of the non-dominant knee flexors on an isokinetic dynamometer. Participants were instructed to contract the knee flexors maximally to resist the dynamometer arm extending from 100° to 10° (0° being horizontal) at $30^{o} \cdot s^{-1}$. After each lengthening contraction participants were instructed to concentrically contract the knee flexors against the dynamometer arm at $60° \cdot s^{-1}$ to move the knee joint back to the starting position. One minutes rest was given between sets. Chen et al. (2011a) has shown this amount of eccentric exercise sufficient enough to induced markers of muscle damage in humans. Visual and verbal encouragement was given throughout the contractions. Following the eccentric exercise participants were asked to refrain from doing any exercise, stretching the hamstring muscles or taking any anti-inflammatory drugs for seven days.

4.1.7 Criterion Measures

Criterion measures consisted of knee flexor MVC at optimal joint angle, plasma CK levels and muscle soreness using a visual analogue scale (VAS). The measurements were taken before (Pre), one hour after (Post), 2 days, 4 days and 7 days following the eccentric exercise. Each testing session was conducted at the same time of the day to ensure that time throughout the day had no influence on the measurements. For comparisons between the sexes MVC was normalized to the pre value to obtain relative MVC. These time periods were chosen in line with previous research to identify peaks in each variable. Chen et al. (2011a) found peaks in soreness and CK occur two and four days following eccentric exercise retrospectively. In addition, other authors have found peaks in force loss occur immediately post or up to two days following the eccentric exercise (McHugh et al., 1999b, Sewright et al., 2008). The criterion measures used within this study (MVC, VAS and CK) have been validated as reliable and valid measures of muscle damage. Chen et al. (2011a) found interclass correlation coeficent values of 0.96 and 1.00 for concentric strength and VAS taken one day and immediately before the eccentric exercise. In addition, Sewright et al. (2008) found significant moderate correlations between MVC loss, sorenees and CK providing evidence that changes within these measures after eccentric exercise are related.

4.1.8 Muscle Soreness

Muscle soreness was assessed using a VAS ranging from 0-10, where 0 represented 'no pain' and 10 representing 'intolerably intense pain', whilst the principle investigator slowly extended the leg at the knee joint from a flexed position (120°) to an extended position (0°) (Chen et al., 2011a, McHugh et al., 1999b). This method is a standardised way of identifying muscle soreness, which is valid and reliable (Chen et al., 2011a).

4.1.9 Plasma Creatine Kinase (CK)

All participants reported to the biochemistry laboratory on five separate occasions (Pre, Post, 2, 4 and 7 days post eccentric exercise). A trained phlebotomist inserted a 21-gauge 25mm ultrathin wall needle (Terumo Medical Corporation, New Jersey, USA) into a superficial forearm vein. A 5-6 mL blood sample was taken using a syringe and stored in serum separator tubes (Becton Dickson and company, Plymouth, UK). If participants were unwilling to give a sample through a superficial forearm vein, they were given the option of blood sampling using the finger tip prick method. After cleaning the area with an alcohol swab, a trained phlebotomist used a high flow lancet (HaeMedic, Ozorków, Poland) to prick the fingertip near the nail bed. Again a 5-6 mL blood sample was taken and stored using serum separator tubes. After being kept on crushed ice for approximately one hour, the blood sample was centrifuged at 0°C for 10 minutes at 4100 rpm (2400 g). Plasma aliquots were then extracted and stored in two separate 1.5 mL eppendorfs at -20^{o}C until further analysis. Plasma CK levels in the serum was determined spectrophotometrically by an automated clinical chemistry analyser (Model ECPK-100) using a test kit (BioAssay Systems, California, USA).

Principle of the test and assay procedure

The Creatine Kinase assay kit is based on enzyme reactions where CK converts Creatine phosphate and ADP to Creatine and ATP. The generated ATP from this reaction is used to phosphorylate glucose by hexokinase to generate glucose-6-phosphate. NADP then oxidizes glucose-6-phosphate in the presence of glucose-6-dehydrogenase. The produced NADP from this reaction (measured at 340 nm) is proportionate to the CK level in the sample.

A 96 microwell plate (BioAssay Systems, California, USA) was used to hold 39 samples, 6 standards of known quantity of CK concentration (ranging from 0.5-1.0 mL) and 2 blank samples (dH_2O and Air), all samples were ran in duplicate. Samples were allowed to thaw

completely before the start of the assay along with the all components of the reagent reconstitution. Firstly, the reagent reconstitution was made by mixing 10 µL substrate solution, 100 µL assay buffer and 1 µL enzyme mix for each individual well. 110 µL of calibrator (10 µL calibrator and 100 µL water) was transferred into a specific pair of calibrator cells at the bottom of a 96-well plate. Following this, 10 µL of each sample was transferred to separate wells in the plate followed by 100 µL of reconstitution reagent. The plate was tapped to mix all components. As CK is fully activated within 20 minutes by glutathione provided in the substrate solution, the plate was incubated at room temperature and read at 20 minutes and again at 40 minutes. The absorbance of each well was measured using a plate reader (Biotek Instruments Inc, Winooski, USA) at 340 nm.

Calculation of results

CK levels in each sample were calculated using the following equation:

$$\text{CK (U/L)} = \frac{OD_{40min} - OD_{20min}}{\text{OD Calibrator} - \text{OD } H_2O} \times 150$$

OD_{40min} and OD_{20min} are OD340nm values at 40min and 20min for the sample. ODcalibrator and ODH20 are OD340nm values of the calibrator and water blank at 40min. The value of 150 is the equivalent activity of the calibrator under the assay conditions. The normal resting measures for CK (U/L) in human serum is 11.0 ± 0.05 based on manufacturers' instructions.

Hemolyzed samples

Hemolysis occurred in some of the serum samples (possibly owing to mechanical trauma of the blood cells, or contact with alcohol on the skin, during the phlebotomy procedure). A systematic linear regression of degree of haemolysis to Optical Density of CK reading was obtained. The data for the regression used a single fresh blood sample (obtained on the day of the CK ELISA run), to which progressively greater amounts of hemolysed blood cells were added. Thus a correction factor for haemolysis on CK data was calculated (to account for the amount of hemolysis in some of the samples) from the slope of the regression of this curve (Figure 9). Previous authors have used this method to account for haemolysis in samples (Koseoglu et al., 2011).

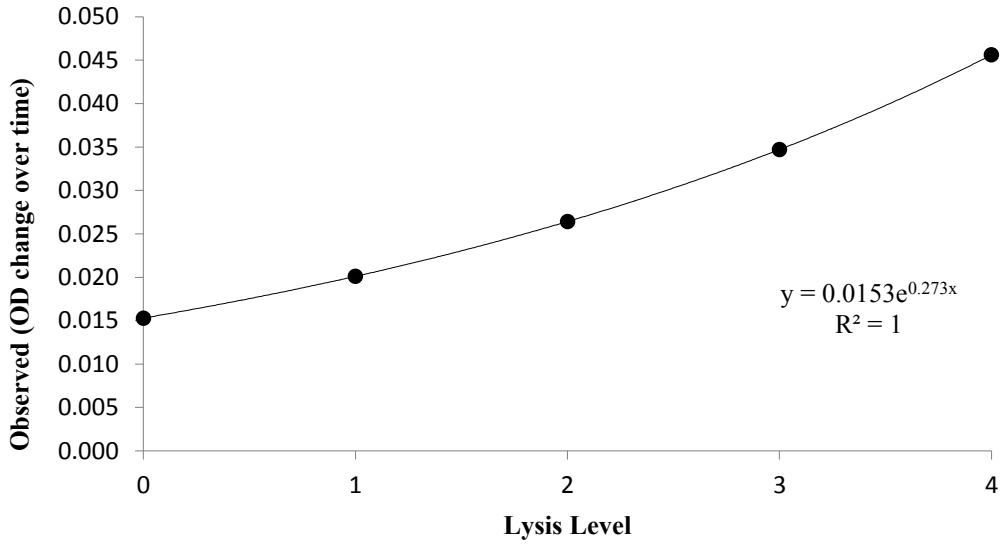

Figure 9. Regression used to calculate correction factor for hemolysis on CK data.

4.2 MEASUREMENT RELIABILITY

A separate group of participants consisting of three males (age 22 ± 0 years, stature 1.75 ± 0.1 m, mass 69.3 ± 3.0kg) and three females (age 22 ± 1.5 years, stature 1.63 ± 0.1 m, mass 68.7 ± 2.0 kg) were recruited for the test-retest reliability of baseline measurements. Range of motion (standing and supine), sit-and-reach score and optimal angle of the knee flexor MVC were repeated at the same time on consecutive days. The inter-day reliability was obtained for all these variables which involved calculating the 95% limits of agreement (± standard deviations of the differences (Bland and Altman, 1986)) between each data set (Figure 10, 11, 12 and 13). Coefficient of variance for ROM (standing and supine), sit and reach score and optimal angle of the knee flexor MVC was 4.1%, 3.8%, 8.1% and 4.8% respectively. The test-retest reliability of these measures was further examined using an intraclass correlation coefficient (r). *R* values for ROM (standing and supine), sit and reach score and optimal angle of the knee flexor MVC was 0.99, 0.98, 0.99 and 0.98 respectively.

Correlation coefficients were also calculated for the flexibility measures. The Pearson product coefficient (r) for ROM (standing and supine) compared with sit and reach score was 0.54 ($p < 0.01$) and 0.72 ($p < 0.01$) respectively and 0.68 ($p < 0.01$) for supine ROM compared with standing ROM. These values show that the flexibility measures were reliable and significantly correlated with one another.

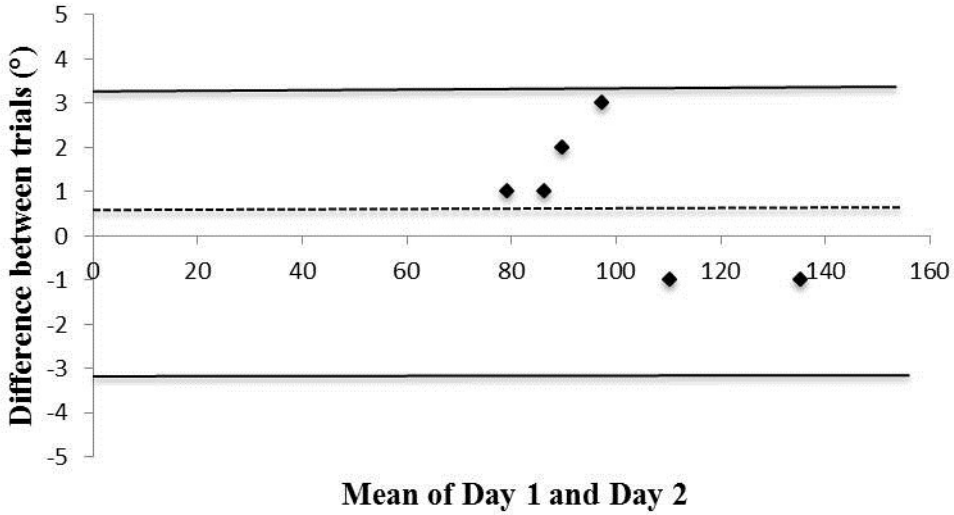

Figure 10. Bland Altman plots for supine range of motion including limits of agreement (±1.96 of the SD) (bold line) and the mean (dashed line).

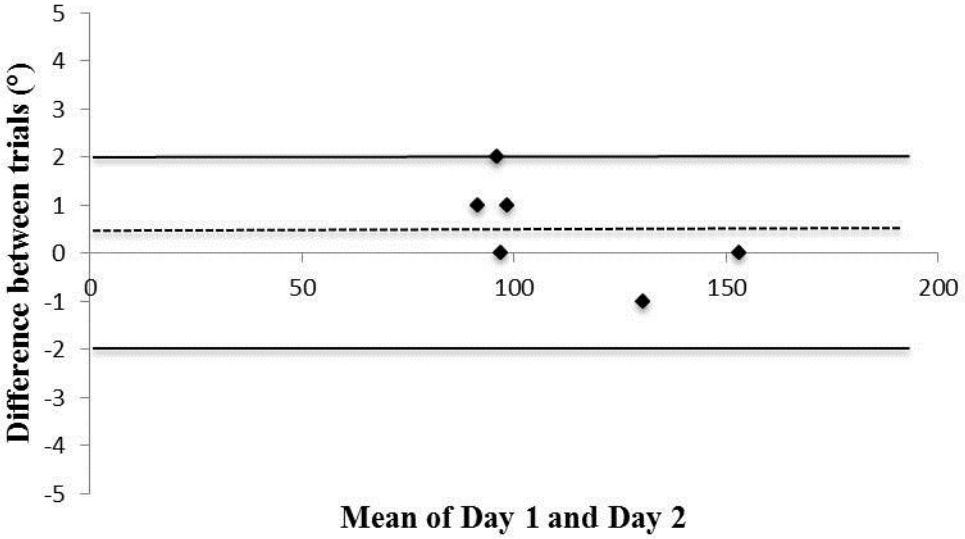

Figure 11. Bland Altman plots for standing range of motion including limits of agreement (±1.96 of the SD) (bold line) and the mean (dashed line).

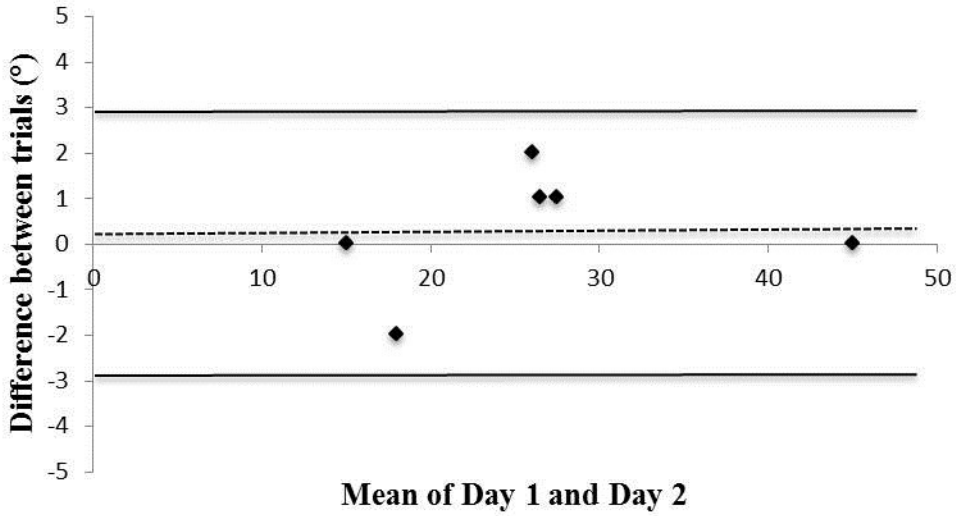

Figure 12. Bland Altman plots for sit and reach including limits of agreement (±1.96 of the SD) (bold line) and the mean (dashed line).

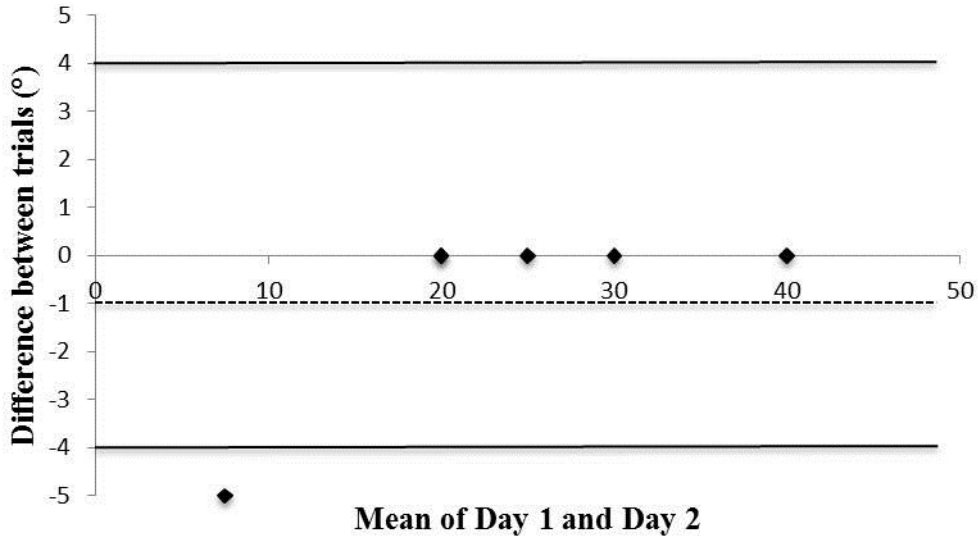

Figure 13. Bland Altman plots for optimal angle of the knee flexor MVC including limits of agreement (±1.96 of the SD) (bold line) and the mean (dashed line).

4.3 STATISTICAL ANALYSES

All data were assessed using SPPS statistic software. Firstly, parametricity of the data was assessed using a Shapiro-Wilk normality test and Levene test for the homogeneity of variance assumption. These tests showed that the data for the baseline measures (MVC, ACSA and flexibility) and two of the criterion measures (MVC and CK) were normally distributed, the cases were independent, and the variance was homogenous. However, these tests also

revealed that the criterion measure soreness was non-parametric. Descriptive data for subject characteristics including MVC, ACSA and flexibility measures (ROM standing and supine, sit and reach score and united weighted flexibility score) were calculated for both groups (Males and Females). Differences at baseline between males and females were tested using independent t-tests for each variable (MVC, ACSA and flexibility).

A repeated measures analysis of variance (ANOVA) (2 × 5) was used to assess the effects of eccentric exercise on the dependent variables (MVC and CK). If a significant interaction effect (sex-by-time) was observed post hoc t-tests with Bonferroni corrections were conducted to compare the dependent variables between males and females at each time point (pre, post, 2, 4 and 7 days post). Non-parametric data (Soreness) was assessed using a Friedmans test for the effect of within group comparisons (Time) and Mann whitneys tests for the between group comparisons (Sex). A Pearsons-product coefficient (r) was conducted between the flexibility measures and criterion measures to determine the effects of flexibility on criterion measures. An alpha level of $p \leq 0.05$ was required for statistical significance.

Power analysis

Power analysis was performed using G*Power to identify a suitable sample size for peak hamstring torque loss following eccentric exercise (Sewright et al., 2008). The data revealed a suggested total sample size of n = 18 (d = 0.7). Based on the peak CK following eccentric exercise in the knee flexors of males and females, there was a suggested total sample size of 4 (d = 6.06). Therefore a participant group of n = 20 was recruited for this study.

Based on post hoc analysis for peak CK revealed that with n = 10 in each groups and based on the means and SD, there was an effect size (d) of 0.55, which is a medium effect (Cohen, 1988).

5. Results

5.1 PARTICIPANT MUSCLE STRUCTURE AND FUNCTIONAL CHARACTERISTICS

Knee flexor MVC and knee flexor anatomical cross sectional area (ACSA) were 67.1% and 44.7% higher in males compared with females, respectively ($P < 0.01$, Table 3). However, when MVC was normalized to ACSA there were no significant differences between the sexes. In addition, no significant differences were observed in flexibility measures between the sexes (Table 2).

Table 2. Gender comparisons of isometric knee flexor torque (MVC), knee flexor anatomical cross sectional area (ACSA), normalized force, sit and reach score, ROM (standing and supine) and optimal angle of the knee flexors.

	Male	Female
MVC (N m)	163.5 ± 42.7	97 ± 20.0*
ACSA (cm^2)	37.7 ± 7.0	26.1 ± 6.4*
Normalized force (N/cm^2)	4.4 ± 1.1	3.9 ± 0.8
Sit and Reach Score (cm)	17.3 ± 7.6	21.0 ± 10.6
Standing ROM (°)	97.9 ± 15.0	101.5 ± 12.6
Supine ROM (°)	98.1 ± 17.1	99.5 ± 14.5
Unit weighted flexibility Z score	-0.4 ± 2.8	0.4 ±2.5
Optimal angle (°)	32.0 ± 8.6	26.0 ± 7.9

Values are presented as mean ± SD
* Denotes a significant difference from males $P < 0.05$

5.2 EFFECTS OF SEX ON MARKERS OF MUSCLE DAMAGE

Muscle strength

Analysis of relative knee flexor MVC torque loss and recovery following maximal eccentric exercise revealed a significant main effect for time ($P < 0.01$) suggesting that the maximal eccentric exercise protocol was sufficient to change the magnitude of knee flexor MVC torque over seven days within the population. However, no significant sex-by-time interaction or main effect for sex were detected, showing no difference between males and females in relative torque loss and recovery (Figure 14). Females experienced a similar maximum MVC loss (32.0 ± 14.5%) compared with males (27.6 ± 12.2%). However, the time point at which the greatest degree of MVC loss was observed was different. Although not significant, males tended to exhibit the greatest amount of MVC loss immediately post eccentric exercise compared with females who exhibited the greatest amount of MVC loss two days following the eccentric exercise. There was no significant difference in rate of recovery of knee flexor MVC torque from peak torque loss in females compared to males. (3.3%/day in females, 2.1%/day in males).

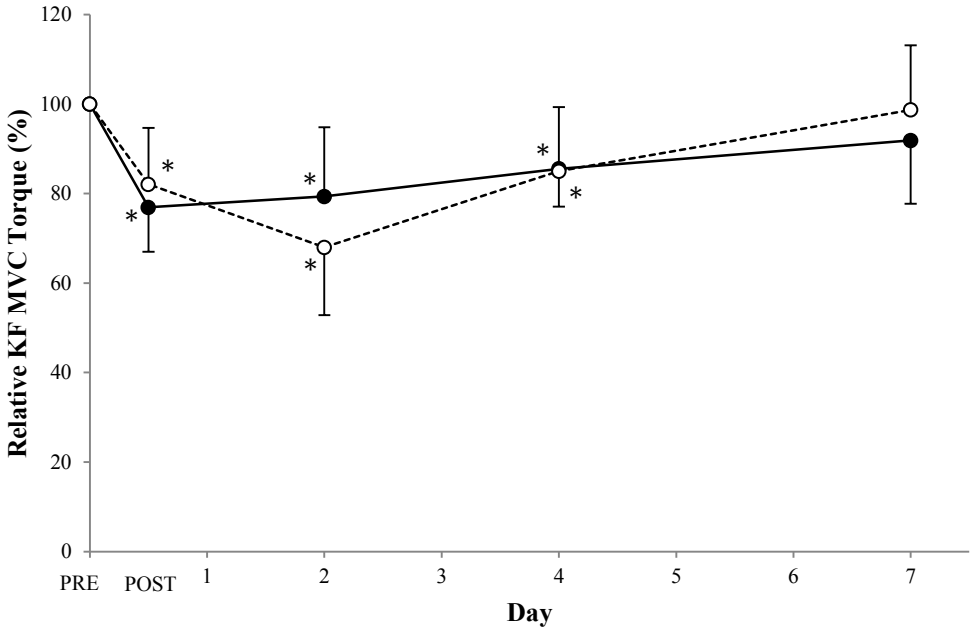

Figure 14. Changes in relative knee flexor MVC torque loss before (Pre), immediately after (Post), 2, 4 and 7 days following a bout of eccentric exercise in males (solid line) and females (dashed line). Data are mean ± SD. * Denotes a significant difference from Pre P<0.05.

Muscle soreness

Following maximal eccentric exercise muscle soreness ratings revealed a significant main effect for time ($P < 0.05$), with max soreness scores of 4.8 ± 1.4 for males and 5.3 ± 2.8 for females (Figure 15). However, no significant sex-by-time interaction was detected throughout the seven days following the eccentric exercise showing no difference in the magnitude of muscle soreness between the sexes.

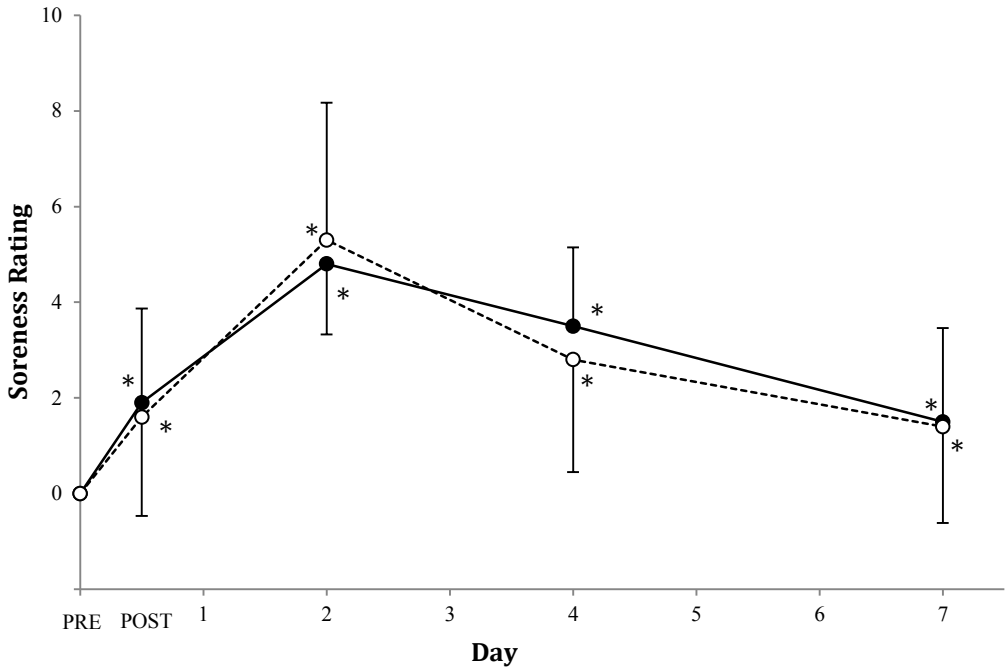

Figure 15. Changes in perceived soreness ratings before (Pre), immediately after (Post), 2, 4 and 7 days following a bout of eccentric exercise in males (solid line) and females (dashed line). Data are mean ± SD. * Denotes a significant difference from Pre P<0.05

Creatine Kinase

After maximal eccentric exercise, a significant ($P < 0.05$), increase in serum CK levels was observed in both males and females showing that the exercise protocol was sufficient to change the circulating levels of serum CK over seven days within the population. However, no significant sex-by-time effect or main effect of sex was observed. Peak CK levels were observed on the fourth day following the eccentric exercise for both males and females (Figure 16).

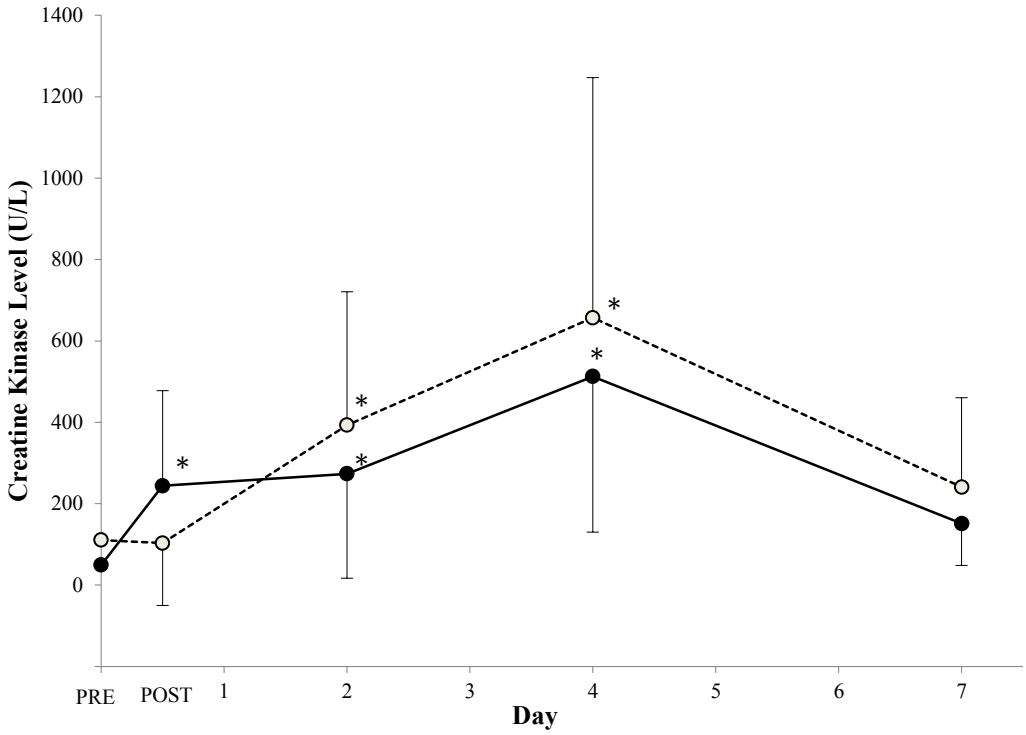

Figure 16. Changes in circulating Creatine Kinase levels before (Pre), immediately after (Post), 2, 4 and 7 days following a bout of eccentric exercise in females (dashed line) and male (solid line) participants. Data are mean ± SD. * Denotes a significant difference from Pre P<0.05

5.3 EFFECTS OF FLEXIBILITY ON MARKERS OF MUSCLE DAMAGE

No significant association was identified between flexibility and any of the criterion measures (knee flexor MVC torque, CK or muscle soreness) when participants were pooled into a single population (Table 3).

Table 3. Correlation (r) between flexibility measures and markers of muscle damage (knee flexor MVC torque, CK levels and soreness rating) when participants were pooled.

	MVC	CK levels	Soreness rating
Standing ROM	0.02	0.12	0.08
Supine ROM	0.01	0.21	0.01
Sit and Reach	0.03	0.10	0.01
Composite flexibility	0.03	0.18	0.03

Further analysis of the effects of flexibility on muscle damage was conducted by dividing the population into a flexible and non-flexible cohort. This was determined using each

participants flexibility Z-score with groups allocated as non-flexible when their Z scores fell under the cohort mean and allocated to flexible when their Z-score fell over the cohort mean. No significant group-by-time interactions were identified between the two groups and any of the criterion markers of muscle damage (knee flexor MVC torque, CK levels or muscle soreness) (Figure 17). These results suggest knee flexor flexibility has no effect on the degree of muscle damage during the seven days following eccentric exercise.

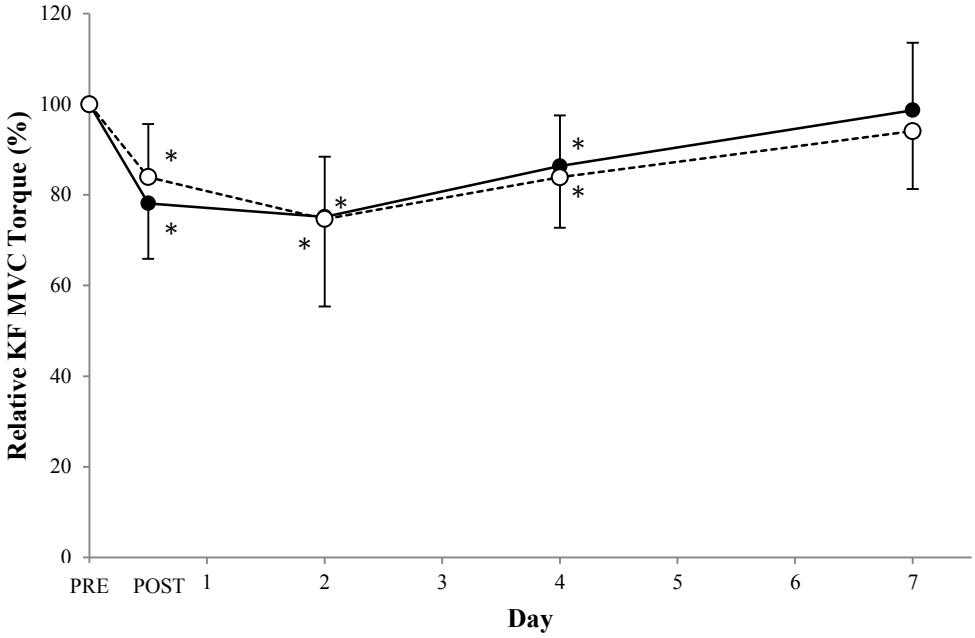

Figure 17. Changes in relative knee flexor MVC torque loss before (Pre), immediately after (Post), 2, 4 and 7 days following a bout of eccentric exercise in flexible (dashed line) and non-flexible (solid line) participants. Data is expressed as mean ± SD. * Denotes a significant difference from Pre P<0.05

6. Discussion

It has previously been established, through animal research, that females have an attenuated response to muscle damage following eccentric exercise compared with males (Amelink and Bar, 1986). However, research within humans remains contentious, possibly owing to a flexibility effect on markers of muscle damage (Chen et al., 2011a, McHugh et al., 1999b) and differences in flexibility within the population used. The aim of this study was to examine the effects of sex and flexibility on indirect markers of muscle damage following a bout of

eccentric exercise. The objective was to consequently determine whether flexibility, sex or an interaction effect, creates the attenuation in markers of muscle damage previously observed amongst females, compared with males. It was hypothesised that lower indices of muscle damage would be observed in females compared with males and there would be a significant negative correlation between muscle damage and flexibility within males and females.

6.1 MAIN FINDINGS

The main findings of this study were 1) there were no significant differences in any of the indirect markers of muscle damage (serum CK levels, knee flexor MVC torque loss and recovery or soreness ratings) following a bout of eccentric exercise between the sexes; 2) there was no significant relationship between knee flexor MTU flexibility and any of the indirect markers of muscle damage (serum CK levels, knee flexor MVC torque loss and recovery or soreness ratings) following a bout of eccentric exercise; 3) there was a trend towards males exhibiting faster damage-induced decrease in strength following eccentric exercise.

The maximal eccentric exercise used within this study was sufficient to induce changes in indices of muscle damage comparable to previous research (Bowers et al., 2004, Chen et al., 2011a, Chen et al., 2011b, Sewright et al., 2008). For example knee flexor torque MVC loss within this study of 30.3 ± 13.3% was comparable to 25.3 ± 2.9% in the knee extensors (Bowers et al., 2004) and 33.0 ± 4.0% in the elbow flexors in previous studies (Chen et al., 2011b). In addition, the knee flexor soreness ratings within this study of 4.8 ± 1.4 in females and 5.3 ± 2.8 in males, were comparable to findings in the elbow flexors of 5.8 ± 2.5 in females and 5.1 ± 2.2 in males (Sewright et al., 2008). Serum CK levels significantly increased from the pre values within this study and peaked on the fourth day following the eccentric exercise (512.6 ± 382.2U/L in males and 657.0 ± 589.5U/L in females), thus reflecting the pattern observed by other authors (Chen et al., 2011a, Sewright et al., 2008). However, the CK values within this study were smaller than other authors, for example, Chen et al. (2011a) found peaks in knee flexor serum CK levels up to 8000U/L in males on the fourth day following a bout of eccentric exercise. Although the CK in the present study is smaller than others, the pattern of muscle damage is comparable to previous research (Chen et al., 2011a, Chen et al., 2011b, Sewright et al., 2008). The smaller absolute CK values in the present study could be attributed to the high variability in CK (Nosaka and Clarkson, 1996), or the participants used. The participants used in the present study were young and moderately active, suggesting they may

perform more eccentric contractions during their daily routine and thus would be expected to be less susceptible to muscle damage (Baird et al., 2012, McHugh et al., 1999b). The results of the present study therefore follow the pattern observed by other authors in torque loss and recovery, CK and soreness ratings following a bout of eccentric exercise.

6.2 EFFECTS OF SEX ON MARKERS OF MUSCLE DAMAGE

In regards to the main findings from the present study, no sex differences in knee flexor markers of muscle damage were observed. These findings do not support the first hypothesis that there would be a significant difference between the sexes in any of the indirect markers of muscle damage following a bout of eccentric exercise. These results suggest that sex has no impact on indirect markers of muscle damage following a bout of eccentric exercise in the knee flexors. Therefore, these findings support that of the human research in the knee extensors (Dannecker et al., 2012, Stupka et al., 2000) and elbow flexors (Sayers and Clarkson, 2001). Stupka et al. (2000) and Sayers and Clarkson (2001) found no significant sex difference in torque loss and recovery following a bout of eccentric exercise in the knee extensors and elbow flexors respectively, thus reflecting the lack of difference in the magnitude of structural damage between the sexes during muscle damage. Furthermore, no significant difference in elbow flexor soreness ratings, serum: myoglobin, tumor necrosis factor, total nitric oxide between the sexes has been observed following eccentric exercise in humans (Dannecker et al., 2012). Differences in direct markers of muscle damage have also been studied between the sexes with findings of no significant difference in elbow flexor Z-line streaming (Stupka et al., 2000). Although no sex difference was observed in the present study, our data is supported by previous data demonstrating no difference in indirect markers (Dannecker et al., 2012, Sayers and Clarkson, 2001, Stupka et al., 2000) and direct markers of muscle damage (Stupka et al., 2000).

The results from the present study contrast some of the human research regarding sex differences in muscle damage (Sewright et al., 2008, Stupka et al., 2001). Sewright et al. (2008) examined the effects of 50 maximal eccentric elbow flexor contractions on indirect markers of muscle damage between males and females. They found males had significantly greater CK activity on the fourth day following the eccentric exercise compared with females. However, they found no other significant differences in any other indirect marker of muscle damage (elbow flexor MVC torque loss and recovery, myoglobin or soreness ratings). Stupka et al.

(2001) also found significantly greater CK levels in males compared with females at every time point throughout the recovery process. Although, they too did not find any significant differences in any of the other markers of muscle damage (knee extensor MVC torque loss and recovery, muscle macrophages or muscle protein content of calpian and conjugated-ubiquitin). Furthermore, Stupka et al. (2001) found no significant difference in Z-line streaming between the sexes, reflecting direct evidence of no differences in structural damage. These results show an attenuation in CK levels in females compared with males, which contrasts the findings of the present study. However, no significant differences have been observed in any other marker of muscle damage, which weakens the hypothesis that males are more susceptible to indices of muscle damage.

As there are no significant sex differences in any other markers of muscle damage other than a trend found in the timing of torque loss, it is conceivable that it is not the extent of the damage which is accentuated in females, rather it is a more effective clearance of CK post damage, or indeed a lower initial release in CK associated with a smaller muscle mass. Van der Meulen (1994) found significantly greater release of CK, aspartate aminotransferase and lactate dehydrogenase, in male rodents compared with females following 1.5 or 2.5 hours of incline treadmill running. Van der Meulen (1994) therefore suggested that part of this greater enzyme release in males could be explained by differences in clearance rates between the sexes. To the authors' knowledge no research has been conducted on the effects of CK clearance rates between the sexes providing an area that needs further investigation. Similarly, it is unknown whether muscle size would have created the greater indices of CK in males compared to females. Males have consistently been shown to have greater baseline CK compared with females (Norton et al., 1985, Sewright et al., 2008). This difference in baseline CK could be due to greater muscle mass in males compared with females. CKs primary function is to facilitate ATP resynthesis within the muscle. Therefore, following eccentric protocols and observing a similar relative torque loss between males and females, it could be assumed that a greater absolute muscle mass is damaged in males, subsequently leading to a greater CK release. Due to the nature of this it maybe important to quantify total CK rather than concentration. In support of this, Glenhill (1988) found higher CK levels in black males compared to white males. The authors suggested that greater muscle mass in black males compared with white males is the cause of the interracial differences in CK. However, some authors have found no significant differences of body composition on baseline levels of CK (Norton et al., 1985). The greater rate of CK clearance and lower muscle mass in females could

therefore explain why previous authors have found an attenuation in markers of muscle damage in females compared with males.

The presence of a sex difference in markers of muscle damage is reported in some of the literature but not others. This is likely to be due to differences between the muscle tendon units measured within these studies, and the attenuation or accentuation of any sex difference. In contrast to the knee flexors used in the present study, the elbow flexors and knee extensors have both demonstrated a sex difference in markers of muscle damage (Sewright et al., 2008, Stupka et al., 2001). The CK response in the elbow flexors is much higher than in the larger muscle groups of the knee extensors and of the knee flexors used in the present study (Peak CK elbow flexors men =10276 women =6594.7, Peak CK knee flexors men = 657.0 women = 512.6), and as such may accentuate any possible sex difference. In addition, Sewright et al. (2008) did not distinguish between oral contraceptive and control females. This may have affected the results as discrepancies in oestrogen have been shown to decrease markers of muscle damage (Thompson et al., 1997). In regards to the sex differences observed in the knee extensors, it is possible that the difference in tendon properties between sexes may influence the relative strain, whereas in the knee flexors (a muscle group in which tendon elastic properties remain unreported) it is likely that the MTU stiffness and thus relative strain is not different between the sexes. Previous authors have established that a greater amount of muscle deformation and thus strain results in greater indices of muscle damage (Lieber, 1993, Song, 2004). Song et al. (2004) found that increasing the amount of strain, by increasing the muscle ROM during eccentric exercise, significantly increased isometric tetanic force loss. This could explain the sex difference in markers of muscle damage observed in the knee extensors, as a significant strain difference would induce greater amounts of muscle damage. Furthermore, it has been suggested that greater stiffness of the tendon in males increases the amount of strain during eccentric contractions (Marginson, 2005). In support of this, Hicks et al. (2013) recently found that change in *Vastus lateralis* fascicle length during eccentric exercise was significantly higher in males compared with females. This would again explain the sex difference seen in the knee extensors. In the knee flexors however, no significant difference in MVC/ACSA between the sexes was observed in the present study and thus it is likely that there would be no difference in eccentric stress and strain as activations levels and ability to perform eccentric exercise are not significantly different (Griffin et al., 1993, Krishnan and Williams, 2009).

In contrast to the results of the present study, animal research has also shown significantly higher indices of muscle damage in male rodents compared with female rodents following a bout of eccentric exercise (Amelink and Bar, 1986, Komulainen et al., 1999, Salminen and Kihlstrom, 1985). For example damage to structural proteins, fiber swelling (Komulainen et al., 1999), CK levels (Amelink and Bar, 1986) and B-gurionidase, an enzyme activated in response to muscle disruption (Salminen and Kihlstrom, 1985), have all been significantly higher in males compared with females. The present study's findings do not support that of the animal research showing a sex difference in markers of muscle damage following eccentric exercise. A possible reason for this could be that the mass of the animal contributes to the attenuation as typically, male rodents weigh significantly more than female rodents (Solleveld et al., 1984). As previously mentioned strain has a negative effect on markers of muscle damage (Lieber, 1993, Song, 2004). This mass difference would create greater indices of stress/strain as a greater load and thus force would be placed on the muscles in males compared with females. Therefore, the greater strain placed on male rodents muscles could explain why there are greater indices of muscle damage in males compared with females. As stated previously, it is unlikely that strain was significantly different between males and females during the eccentric protocol in the present study thus explaining why the present study's research does not support the animal research findings.

Another possible reason for the findings of the greater damage experienced by male animals compared to female animals, could be due to the duration and mode of the damage protocol used in animal studies in comparison to the exercise used within the present study. The animal research mainly used downhill treadmill running as their mode of damage; for example 96 hours of downhill running (Komulainen et al., 1999). This mode of exercise differs significantly from the six sets of 10 eccentric contractions used in the current study. Downhill running would require the recruitment of many muscle groups compared to the one focus muscle group in the present study, and most of the human studies investigating sex differences in muscle damage (Dannecker et al., 2012, Sayers and Clarkson, 2001, Sewright et al., 2008, Stupka et al., 2000). The recruitment of many muscle groups would result in far greater indices of muscle damage compared to the present study as more muscles are being damaged simultaneously. The duration of the damage protocol in animal studies also differs significantly from that used in the present study; for example 96 hours compared to approximately 12 minutes. The duration and mode of the damage protocol used in animal studies would have produced excessive amounts of muscle damage compared to that seen in the present study. It

could be suggested that sex differences in markers of muscle damage may only be detectable when excessive exercise is used. As this study only used 60 eccentric contractions it is possible that this was not enough to induce the sex difference seen by the animal research (Amelink and Bar, 1986, Komulainen et al., 1999, Salminen and Kihlstrom, 1985). The mode and duration of the exercise used in animal studies could explain why they observe a sex difference compared to the present study.

6.3 THE EFFECTS OF FLEXIBILITY ON MARKERS OF MUSCLE DAMAGE

The second main finding of this study was no significant relationship between MTU flexibility and markers of muscle damage. These findings are in contrast to the second hypothesis that there would be a significant relationship between MTU flexibility and markers of muscle damage. The results make it evident that flexibility cannot be an explanation of the sex differences in muscle damage previously hypothesized. The findings of the present study do not support the findings of prior flexibility research showing a significant attenuation in markers of muscle damage in more compliant knee flexor muscles (Chen et al., 2011a, McHugh et al., 1999b). McHugh et al. (1999b) examined the effects of knee flexor MTU compliance on markers of muscle damage following a bout of eccentric exercise and found the compliant group had a significant attenuation in isometric knee flexor strength, pain, muscle tenderness and CK activity. However, there were sex bias groupings in the study conducted by McHugh et al. (1999b) study (mostly female participants in the flexible group, mostly males in the non-flexible group) making it difficult to separate the influence of flexibility from the influence of sex. In addition to this, Chen et al. (2011a) found following eight weeks of PNF flexibility training there was a significant decrease in markers of muscle damage (CK activity, isometric force loss and perceived muscle soreness) compared with an age matched control group. This finding was attributed to a concomitant shift in optimal angle of knee flexors. Chen et al. (2011a) suggested the shift in optimal angle towards a longer muscle length was due to an increase in sarcomeres in series and this was the fundamental difference between flexibility and non-flexibility trained individuals that created the attenuation in markers of muscle damage. With all else being equal, an increase in sarcomeres in series would result in less sarcomeres operating on the descending limb of the length tension relationship and thus less damage (Morgan, 1990).

The present study's findings do not support the flexibility research showing an inverse relationship between muscle compliance and markers of muscle damage. This could be due to a limited array of ROM within the participants in this study in comparison to previous research. The participants knee flexor ROM following flexibility training was significantly different between the groups (Control 99.0°, static stretching 120.1° and PNF 123.1° (Chen et al., 2011a); however, there was no significant difference in flexibility between the flexibility groups in the present study. Participants within this study were required to undertake three different ROM measurements (sit and reach, standing ROM and supine ROM). Even though there was a variety in the cohorts' flexibility, it was not significantly different between the flexibility groups, when split based on cohort mean flexibility. Thus, the difference in flexibility may not have been large enough to induce an attenuation effect similar to that seen in the knee flexors (Chen et al., 2011a, McHugh et al., 1999b). Additionally, there was no difference in flexibility between the sexes, which could explain the lack of significant difference between the sexes in markers of muscle damage. However, with this being said no relationship between MTU flexibility within the cohort and any of the markers of muscle damage was identified regardless of sex. Therefore, it seems unlikely that the lack of natural difference in flexibility within the cohort, or between the sexes, could cause the absence of no significant difference in markers of muscle damage.

The suggestion proposed by Chen et al (2011a), that the difference in optimal angle creates the attenuation in markers of muscle damage, could provide further reason for the difference in findings of the present study to previous research (Chen et al., 2011a). There was no significant difference in optimal angle of force generation in the flexible and non-flexible groups in the present study. In contrast Chen et al. (2011a) found a significant difference in optimal angle in the PNF group compared to the control group. It can therefore be suggested that a shift in optimal angle towards a longer muscle length could be the sole reason for an attenuation in markers of muscle damage previously observed. Furthermore, it is possible that this shift in optimal angle can only be seen following flexibility training and not in natural differences in flexibility used in the present study. It could therefore be hypothesized that increased flexibility through flexibility training creates an attenuation in muscle damage through the increase in sarcomeres in series. However, no significant attenuation in markers of muscle damage occurs for those who are naturally more flexible. In further support of this theory, there were no significant differences in optimal angle between the sexes within this study, which again, could

provide a reason as to why there was no significant difference in markers of muscle damage between the sexes.

Furthermore, fundamental limitations and differences within the research by McHugh et al. (1999b) and Chen et al. (2011a) could explain the contrast between the current study's findings and theirs. For example, it is possible that sex acted as a confounding variable within McHugh et al. (1999b) research as sex bias groups were used. Therefore it is impossible to suggest that passive stiffness of the muscle was the only variable that contributed to the compliance difference in markers of muscle damage. In addition the vaidity and reliability of the work by McHugh et al. (1999b) could be questioned as they only measured CK in 12 of the participants (as opposed to the 20 that contributed to the full story), reducing the power of the study, and the participants in the compliant group appeared to recover on average to 115% of their baseline isometric strength on the third day following the eccentric exercise. Even though this is plausible, as overcompensation does occur following injury, it is unlikely that all the participants would have recovered to such a high percentage of their baseline isometric strength so quickly (i.e. three days). Isometric strength has been consistently shown to completely recover anywhere from five to eight days following the eccentric exercise (Bowers et al., 2004, Sewright et al., 2008, Stupka et al., 2001) with some authors observing a second drop in isometric strength 24 hours following the exercise (MacIntyre et al., 1995).

Furthermore, Chen et al. (2011a) study was a flexibility training study in which the participants undertook eight weeks of PNF or static stretching. The longitudinal adaptations in flexibility training may be completely different to the determinants of a sex difference and this may explain why the findings in the knee flexors contrast the findings of this study. For example flexibility-training studies have been shown to increase the fascicle size in relation to the muscle length (Kuno-Mizumura, 2007). This increase in fascicle size in relation to muscle length could explain the attenuation in markers of muscle damage seen in the flexibility-trained participants in the knee flexors (Chen et al., 2011a). Furthermore, flexibility-training studies have shown an increase in optimal angle towards a longer muscle length (Chen et al., 2011a). This increase in optimal angle towards a longer muscle length may explain the attenuation in markers of muscle damage in flexibility-trained individuals as according to Morgan's (1990) popping sarcomere theory the greater the amount of sarcomeres working on the descending limb of the length tension relationship the greater the muscle disruption. As the current study compared differences in MTU flexibility in a cohort who had no previous flexibility training, it

is unlikely that fascicle size in relation to muscle length and optimal angle of force production, would have been significantly different amongst the cohort and thus would not have affected the results. Furthermore, it is plausible that the eight weeks of flexibility training in the study by Chen et al. (2011a) would have induced some muscle damage and may have stimulated the repeated bout effect. The repeated bout effect is the name given to the attenuation in markers of muscle damage following a second bout of exercise (Nosaka and Clarkson, 1995). It is therefore possible that the eight weeks of training would have attenuated the indirect markers of muscle damage in comparison to the increase in flexibility.

A possible limitation of the present study was the use of indirect markers of muscle damage instead of direct markers. It is well known that direct markers of muscle damage are the gold standard verification of muscle damage making them the preferred method of identifying muscle damage (Friden et al., 1981). Indirect markers of muscle damage such as torque loss and recovery have been criticized due to the fact that pain, rather that direct disruption of contractile material, can result in decreased MVC torque. However, indirect markers of muscle damage such as those used within this study (CK levels, knee flexor MVC torque loss and recovery and soreness ratings) are well-established reliable and valid indicators of the extent of damage that occurs within the muscle (McHugh et al., 1999a, McHugh et al., 1999b, Morton et al., 2005). In addition, torque loss and recovery provides a meaningful indication of muscle damage with regards to the functional performance in comparison to some direct markers of muscle damage. Furthermore, indirect markers where chosen as direct markers involve more invasive procedures such as biopsies creating further limitations to the study.

6.4 FUTURE RECOMMENDATIONS

Future studies should consider the impact of oestrogen manipulation on markers of muscle damage for example the oral contraceptive pill. Oestrogen is known to have significant effects on the viscoelastic properties of the MTU (Hansen et al., 2009) and the suppression of oestrogen through use of the oral contraceptive pill may provide evidence as to why females have an attenuated response to EIMD in previous literature. In addition, further muscles should be studied as the knee flexors made the measurement of the MTU with ultrasound impossible. It may be beneficial in future work to identify using ultrasound, the distinctions between the muscle and tendon as they are known to act differently under stretch (Morse et al., 2008).

Different muscles would give a better overall picture of the effects of MTU stiffness, flexibility and stress/strain being applied during the eccentric contractions on markers of EIMD.

6.5 CONCLUSION

In conclusion, the main findings of the present investigation were no significant differences between the sexes in markers of muscle damage and no significant relationship between knee flexor MTU flexibility and markers of muscle damage. The findings of this study may not be relevant to any other muscle group apart from the knee flexors as MTU architecture may have an effect on markers of muscle damage. The findings indicate that natural differences in flexibility such as that seen between the sexes has no attenuation effect on functional parameters following eccentric exercise. The reason there is no significant difference in markers of muscle damage between the sexes could be solely due to the amount of stress/strain applied on the muscle throughout the eccentric exercise. Previous authors may have observed a difference due to differences in stress/strain applied through the muscle depending on muscle group, eccentric protocol, muscle size and muscle structure between the sexes. Further research is therefore needed on the differences in stress/strain between the sexes and its effects on markers of muscle damage.

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